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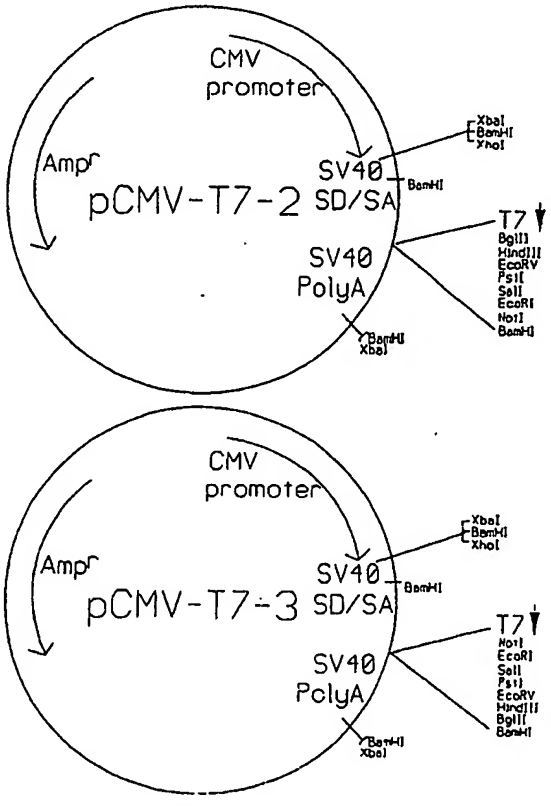
(54) Title: HUMAN NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR COMPOSITIONS AND METHODS EMPLOYING SAME

(57) Abstract

Nucleic acids encoding human neuronal nicotinic acetylcholine receptor alpha and beta subunits, mammalian and amphibian cells containing said nucleic acids, methods for producing alpha and beta subunits and recombinant (i.e., isolated or substantially pure) alpha subunits (specifically α_4 and α_7) and beta subunits (specifically β_4) are provided. In addition, combinations of subunits (i.e., α_1, α_2, α_3, α_4, and/or α_7 subunits in combination with β_4 subunits; or β_2, β_3 and/or β_4 subunits in combination with α_4 and/or α_7 subunits) are provided.



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HUMAN NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR COMPOSITIONS AND METHODS EMPLOYING SAME

This invention relates to nucleic acids encoding human neuronal nicotinic acetylcholine receptor protein subunits, as well as the proteins themselves. In particular, human neuronal nicotinic acetylcholine receptor α-subunit-encoding nucleic acids, α-subunit proteins, β-subunit-encoding nucleic acids, β-subunit proteins, and combinations thereof are provided.

BACKGROUND OF THE INVENTION

Ligand-gated ion channels provide a means for communication between cells of the central nervous system. These channels convert a signal (e.g., a chemical referred to as a neurotransmitter) that is released by one cell into an electrical signal that propagates along a target cell membrane. A variety of neurotransmitters and neurotransmitter receptors exist in the central and peripheral nervous systems. Five families of ligand-gated receptors, including the nicotinic acetylcholine receptors (NAChRs) of neuromuscular and neuronal origins, have been identified (Stroud et al. (1990) Biochemistry 29:11009-11023). There is, however, little understanding of the manner in which the variety of receptors generates different responses to neurotransmitters or to other modulating ligands in different regions of the nervous system.

The nicotinic acetylcholine receptors (NAChRs) are multisubunit protein of neuromuscular and neuronal origins. These receptors form ligand-gated ion channels that mediate synaptic transmission between nerve and muscle and between neurons upon interaction with the neurotransmitter acetylcholine (ACh). Since various nicotinic acetylcholine receptor (NAChR) subunits exist, a variety of NAChR compositions (i.e., combinations of

subunits) exist. The different NAChR compositions exhibit different specificities for various ligands and are thereby pharmacologically distinguishable. Thus, the nicotinic acetylcholine receptors expressed at the vertebrate neuromuscular junction in vertebrate sympathetic ganglia and in the vertebrate central nervous system have been distinguished on the basis of the effects of various ligands that bind to different NAChR compositions. For example, the elapid α-neurotoxins that block activation of nicotinic acetylcholine receptors at the neuromuscular junction do not block activation of some neuronal nicotinic acetylcholine receptors that are expressed on several different neuron-derived cell lines.

Muscle NAChR is a glycoprotein composed of five subunits with the stoichiometry $\alpha_2\beta(\gamma \text{ or } \epsilon)\delta$. Each of the subunits has a mass of about 50-60 kilodaltons (kd) and is encoded by a different gene. The $\alpha_2\beta(\gamma \text{ or } \epsilon)\delta$ complex forms functional receptors containing two ligand binding sites and a ligand-gated transmembrane channel. Upon interaction with a cholinergic agonist, muscle nicotinic AChRs conduct sodium ions. The influx of sodium ions rapidly short-circuits the normal ionic gradient maintained across the plasma membrane, thereby depolarizing the membrane. By reducing the potential difference across the membrane, a chemical signal is transduced into an electrical signal that signals muscle contraction at the neuromuscular junction.

Functional muscle nicotinic acetylcholine receptors have been formed with $\alpha\beta\delta\gamma$ subunits, $\alpha\beta\gamma$ subunits, $\alpha\beta\delta$ subunits, $\alpha\delta\gamma$ subunits or $\alpha\delta$ subunits, but not with only one subunit (see e.g., Kurosaki et al. (1987) FEBS Lett. 214: 253-258; Camacho et al. (1993) J. Neuroscience 13:605-613). In contrast, functional neuronal AChRs (nAChRs) can be formed from α subunits alone or combinations of α and β subunits. The larger α

subunit is generally believed to be the ACh-binding subunit and the lower molecular weight β subunit is generally believed to be the structural subunit, although it has not been definitively demonstrated that the β subunit does not have the ability to bind ACh. Each of the subunits which participate in the formation of a functional ion channel are, to the extent they contribute to the structure of the resulting channel, "structural" subunits, regardless of their ability (or inability) to bind ACh. Neuronal AChRs (nAChRs), which are also ligand-gated ion channels, are expressed in ganglia of the autonomic nervous system and in the central nervous system (where they mediate signal transmission), in post-synaptic locations (where they modulate transmission), and in pre- and extra-synaptic locations (where they may have additional functions).

Nucleic acids encoding NAChRs has been isolated from several sources. Based on the information available from such work, it has been evident for some time that NAChRs expressed in muscle, in autonomic ganglia, and in the central nervous system are functionally diverse. This functional diversity could be due, at least in part, to the large number of different NAChR subunits which exist. There is an incomplete understanding, however, of how (and which) NAChR subunits combine to generate unique NAChR subtypes, particularly in neuronal cells. Indeed, there is evidence that only certain NAChR subtypes may be involved in diseases such as Alzheimer's disease. Moreover, it is not clear whether NAChRs from analogous tissues or cell types are similar across species.

Accordingly, there is a need for the isolation and characterization of nucleic acids encoding each human neuronal NAChR subunit, recombinant cells containing such subunits and receptors prepared therefrom. In order to study the function of human neuronal AChRs and to obtain

disease-specific pharmacologically active agents, there is also a need to obtain isolated (preferably purified) human neuronal nicotinic AChRs, and isolated (preferably purified) human neuronal nicotinic AChR subunits. In addition, there is also a need to develop assays to identify such pharmacologically active agents.

The availability of such nucleic acids, cells, receptor subunits and receptor compositions will eliminate the uncertainty of speculating as to human nNAChR structure and function based on predictions drawn from non-human nNAChR data, or human or non-human muscle or ganglia NAChR data.

Therefore, it is an object herein to isolate and characterize nucleic acids encoding subunits of human neuronal nicotinic acetylcholine receptors. It is also an object herein to provide methods for recombinant production of human neuronal nicotinic acetylcholine receptor subunits. It is also an object herein to provide purified receptor subunits and to provide methods for screening compounds to identify compounds that modulate the activity of human neuronal AChRs.

These and other objects will become apparent to those of skill in the art upon further study of the specification and claims.

BRIEF DESCRIPTION OF THE INVENTION

In accordance with the present invention, there are provided isolated nucleic acids encoding novel human alpha and beta subunits of neuronal NAChRs. In particular, isolated DNA encoding human α_4, α_7, and β_4 subunits of neuronal NAChRs are provided. Messenger RNA and polypeptides encoded by the above-described nucleic acids are also provided.

Further in accordance with the present invention, there are provided recombinant human neuronal nicotinic AChR subunits, including α_4, α_7, and β_4 subunits, as well as methods for the production thereof. In addition, recombinant human neuronal nicotinic acetylcholine receptors containing at least one human neuronal nicotinic AChR subunit are also provided, as well as methods for the production thereof. Further provided are recombinant neuronal nicotinic AChRs that contain a mixture of one or more NAChR subunits encoded by a host cell, and one or more nNAChR subunits encoded by heterologous DNA or RNA (i.e., DNA or RNA as described herein that has been introduced into the host cell), as well as methods for the production thereof.

Plasmids containing DNA encoding the above-described subunits are also provided. Recombinant cells containing the above-described DNA, mRNA or plasmids are also provided herein. Such cells are useful, for example, for replicating DNA, for producing human NAChR subunits and recombinant receptors, and for producing cells that express receptors containing one or more human subunits.

Also provided in accordance with the present invention are methods for identifying cells that express functional nicotinic acetylcholine receptors. Methods for identifying compounds which modulate the activity of NAChRs are also provided.

The DNA, mRNA, vectors, receptor subunits, receptor subunit combinations and cells provided herein permit production of selected neuronal nicotinic AChR subunits and specific combinations thereof, as well as antibodies to said receptor subunits. This provides a means to prepare synthetic or recombinant receptors and receptor subunits that are substantially free of

contamination from many other receptor proteins whose presence can interfere with analysis of a single NAChR subunit. The availability of desired receptor subunits makes it possible to observe the effect of a drug substance on a particular receptor subtype and to thereby perform initial *in vitro* screening of the drug substance in a test system that is specific for humans and specific for a human neuronal nicotinic AChR subtype.

The availability of subunit-specific antibodies makes possible the application of the technique of immunohistochemistry to monitor the distribution and expression density of various subunits (e.g., in normal vs diseased brain tissue). Such antibodies could also be employed for diagnostic and therapeutic applications.

The ability to screen drug substances *in vitro* to determine the effect of the drug on specific receptor compositions should permit the development and screening of receptor subtype-specific or disease-specific drugs. Also, testing of single receptor subunits or specific receptor subunit combinations with a variety of potential agonists or antagonists provides additional information with respect to the function and activity of the individual subunits and should lead to the identification and design of compounds that are capable of very specific interaction with one or more receptor subtypes. The resulting drugs should exhibit fewer unwanted side effects than drugs identified by screening with cells that express a variety of subtypes.

Further in relation to drug development and therapeutic treatment of various disease states, the availability of nucleic acids encoding human nNAChR subunits enables identification of any alterations in such genes (e.g., mutations) which may correlate with the occurrence of certain disease states. In addition, the

creation of animal models of such disease states becomes possible, by specifically introducing such mutations into synthetic DNA sequences which can then be introduced into laboratory animals or *in vitro* assay systems to determine the effects thereof.

BRIEF DESCRIPTION OF THE FIGURE

Figure 1 presents a restriction map of two pCMV promoter-based vectors, pCMV-T7-2 and pCMV-T7-3.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, we have isolated and characterized nucleic acids encoding novel human alpha and beta subunits of neuronal NAChRs. Specifically, isolated DNAs encoding human α_4, α_7, and β_4 subunits of neuronal NAChRs are described herein. Recombinant messenger RNA (mRNA) and recombinant polypeptides encoded by the above-described nucleic acids are also provided.

As used herein, isolated (or substantially pure) as a modifier of DNA, RNA, polypeptides or proteins means that the DNA, RNA, polypeptides or proteins so designated have been separated from their *in vivo* cellular environments through the efforts of human beings. Thus as used herein, isolated (or substantially pure) DNA refers to DNAs purified according to standard techniques employed by those skilled in the art (see, e.g., Maniatis et al.(1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY).

Similarly, as used herein, "recombinant" as a modifier of DNA, RNA, polypeptides or proteins means that the DNA, RNA, polypeptides or proteins so designated have

been prepared by the efforts of human beings, e.g., by cloning, recombinant expression, and the like. Thus as used herein, recombinant proteins, for example, refers to proteins produced by a recombinant host, expressing nucleic acidss which have been added to that host through the efforts of human beings.

As used herein, a human alpha subunit gene is a gene that encodes an alpha subunit of a human neuronal nicotinic acetylcholine receptor. The alpha subunit is a subunit of the NAChR to which ACh binds. Assignment of the name "alpha" to a putative nNAChR subunit, according to Deneris et al. [Tips (1991) 12:34-40] is based on the conservation of adjacent cysteine residues in the presumed extracellular domain of the subunit that are the homologues of cysteines 192 and 193 of the *Torpedo* alpha subunit (see Noda et al. (1982) Nature 299:793-797). As used herein, an alpha subunit refers to a human nNAChR subunit that is encoded by nucleic acids that hybridizes under high stringency conditions to at least one of the nNAChR alpha subunit-encoding nucleic acidss (or deposited clones) disclosed herein. An alpha subunit also binds to ACh under physiological conditions and at physiological concentrations and, in the optional presence of a beta subunit (i.e., some alpha subunits are functional alone, while others require the presence of a beta subunit), generally forms a functional AChR as assessed by methods described herein or known to those of skill in this art.

Also contemplated are alpha subunits encoded by nucleic acids that encode alpha subunits as defined above, but that by virtue of degeneracy of the genetic code do not necessarily hybridize to the disclosed nucleic acids or deposited clones under specified hybridization conditions. Such subunits also participate in the formation of a functional receptor, as assessed by

the methods described herein or known to those of skill in the art, generally with one or more beta subunits. Typically, unless an alpha subunit is encoded by RNA that arises from alternative splicing (i.e., a splice variant), alpha-encoding nucleic acids and the alpha subunit encoded thereby share substantial sequence homology with at least one of the alpha subunit nucleic acidss (and proteins encoded thereby) described or deposited herein. It is understood that DNA or RNA encoding a splice variant may overall share less than 90% homology with the DNA or RNA provided herein, but include regions of nearly 100% homology to a DNA fragment or deposited clone described herein, and encode an open reading frame that includes start and stop codons and encodes a functional alpha subunit.

As used herein, a splice variant refers to variant NAChR subunit-encoding nucleic acid(s) produced by differential processing of primary transcript(s) of genomic DNA, resulting in the production of more than one type of mRNA. cDNA derived from differentially processed genomic DNA will encode NAChR subunits that have regions of complete amino acid identity and regions having different amino acid sequences. Thus, the same genomic sequence can lead to the production of multiple, related mRNAs and proteins. Both the resulting mRNAs and proteins are referred to herein as "splice variants".

Stringency of hybridization is used herein to refer to conditions under which polynucleic acid hybrids are stable. As known to those of skill in the art, the stability of hybrids is reflected in the melting temperature (T_m) of the hybrids. T_m can be approximated by the formula:

$$81.5°C - 16.6(\log_{10}[Na^+]) + 0.41(\%G+C) - 600/l,$$

where l is the length of the hybrids in nucleotides. T_m decreases approximately 1-1.5°C with every 1% decrease in sequence homology. In general, the stability of a hybrid is a function of sodium ion concentration and temperature. Typically, the hybridization reaction is performed under conditions of lower stringency, followed by washes of varying, but higher, stringency. Reference to hybridization stringency relates to such washing conditions. Thus, as used herein:

(1) HIGH STRINGENCY refers to conditions that permit hybridization of only those nucleic acid sequences that form stable hybrids in 0.018M NaCl at 65°C (i.e., if a hybrid is not stable in 0.018M NaCl at 65°C, it will not be stable under high stringency conditions, as contemplated herein). High stringency conditions can be provided, for example, by hybridization in 50% formamide, 5X Denhardt's solution, 5X SSPE, 0.2% SDS at 42°C, followed by washing in 0.1X SSPE, and 0.1% SDS at 65°C;

(2) MODERATE STRINGENCY refers to conditions equivalent to hybridization in 50% formamide, 5X Denhardt's solution, 5X SSPE, 0.2% SDS at 42°C, followed by washing in 0.2X SSPE, 0.2% SDS, at 65°C; and

(3) LOW STRINGENCY refers to conditions equivalent to hybridization in 10% formamide, 5X Denhardt's solution, 6X SSPE, 0.2% SDS, followed by washing in 1X SSPE, 0.2% SDS, at 50°C.

It is understood that these conditions may be duplicated using a variety of buffers and temperatures and that they are not necessarily precise.

Denhardt's solution and SSPE (see, e.g., Sambrook, Fritsch, and Maniatis, in: Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1989) are well known to those of skill in the art as are other suitable hybridization buffers. For example, SSPE is pH 7.4 phosphate-buffered 0.18M NaCl. SSPE can be prepared, for example, as a 20X stock solution by dissolving 175.3 g of NaCl, 27.6 g of NaH_2PO_4 and 7.4 g EDTA in 800 ml of water, adjusting the pH to 7.4, and then adding water to 1 liter. Denhardt's solution (see, Denhardt (1966) Biochem. Biophys. Res. Commun. 23:641) can be prepared, for example, as a 50X stock solution by mixing 5 g Ficoll (Type 400, Pharmacia LKB Biotechnology, INC., Piscataway NJ), 5 g of polyvinylpyrrolidone, 5 g bovine serum albumin (Fraction V; Sigma, St. Louis MO) water to 500 ml and filtering to remove particulate matter.

The phrase "substantial sequence homology" is used herein in reference to the nucleotide sequence of DNA, the ribonucleotide sequence of RNA, or the amino acid sequence of protein, that has slight and non-consequential sequence variations from the actual sequences disclosed herein. Species having substantial sequence homology are considered to be equivalent to the disclosed sequences and as such are within the scope of the appended claims. In this regard, "slight and non-consequential sequence variations" mean that "homologous" sequences, i.e., sequences that have substantial homology with the DNA, RNA, or proteins disclosed and claimed herein, are functionally equivalent to the sequences disclosed and claimed herein. Functionally equivalent sequences will function in substantially the same manner

to produce substantially the same compositions as the nucleic acid and amino acid compositions disclosed and claimed herein. In particular, functionally equivalent nucleic acidss encode proteins that are the same as those disclosed herein or that have conservative amino acid variations, such as substitution of a non-polar residue for another non-polar residue or a charged residue for a similarly charged residue. These changes include those recognized by those of skill in the art as those that do not substantially alter the tertiary structure of the protein.

In practice, the term substantially the same sequence means that DNA or RNA encoding two proteins hybridize under conditions of high stringency and encode proteins that have the same sequence of amino acids or have changes in sequence that do not alter their structure or function. As used herein, substantially identical sequences of nucleotides share at least about 90% identity, and substantially identical amino acid sequences share more than 95% amino acid identity. It is recognized, however, that proteins (and DNA or mRNA encoding such proteins) containing less than the above-described level of homology arising as splice variants or that are modified by conservative amino acid substitutions (or substitution of degenerate codons) are contemplated to be within the scope of the present invention.

As used herein, "α_4 subunit DNA" refers to DNA encoding a neuronal nicotinic acetylcholine receptor subunit of the same name. Such DNA can be characterized in a number of ways, for example

said DNA may encode the amino acid sequence set forth in SEQ. ID No. 6, or

said DNA may encode the amino acid sequence encoded by clone HnAChRα4.2, deposited under ATCC Accession No. 69239, or

the 5' nucleotides of said DNA may encode the amino acid sequence encoded by clone HnAChRα4.1, deposited under ATCC Accession No. 69152.

Presently preferred α_4-encoding DNAs can be characterized as follows

said DNA may hybridize to the coding sequence set forth in SEQ. ID No. 5 (preferably to substantially the entire coding sequence thereof, i.e., nucleotides 173-2056) under high stringency conditions, or

said DNA may hybridize under high stringency conditions to the sequence (preferably to substantially the entire sequence) of the α_4-encoding insert of clone HnAChRα4.2, deposited under ATCC Accession No. 69239, or

the 5' nucleotides of said DNA may hybridize under high stringency conditions to the sequence of the α_4-encoding insert of clone HnAChRα4.1, deposited under ATCC Accession No. 69152.

Especially preferred α_4-encoding DNAs of the invention are characterized as follows

DNA having substantially the same nucleotide sequence as the coding region set forth in SEQ. ID No. 5 (i.e., nucleotides 173-2056 thereof), or

DNA having substantially the same nucleotide sequence as the α_4-encoding insert of clone HnAChRα4.2, deposited under ATCC Accession No. 69239, or

the 5' nucleotides of said DNA have substantially the same sequence as the α_4-encoding insert of clone HnAChRα4.1, deposited under ATCC Accession No. 69152.

Typically, unless an α_4 subunit arises as a splice variant, α_4-encoding DNA will share substantial

sequence homology (i.e., greater than about 90%), with the α_4 DNAs described or deposited herein. DNA or RNA encoding a splice variant may share less than 90% overall sequence homology with the DNA or RNA provided herein, but such a splice variant would include regions of nearly 100% homology to the above-described DNAs.

As used herein, "α_7 subunit DNA" refers to DNA encoding a neuronal nicotinic acetylcholine receptor subunit of the same name. Such DNA can be characterized in a number of ways, for example, the nucleotides of said DNA may encode the amino acid sequence set forth in SEQ. ID No. 8. Presently preferred α_7-encoding DNAs can be characterized as DNA which hybridizes under high stringency conditions to the coding sequence set forth in SEQ. ID No. 7 (preferably to substantially the entire coding sequence thereof, i.e., nucleotides 73-1581). Especially preferred α_7-encoding DNAs of the invention are characterized as having substantially the same nucleotide sequence as the coding sequence set forth in SEQ. ID No. 7 (i.e., nucleotides 73-1581 thereof).

Typically, unless an α_7 subunit arises as a splice variant, α_7-encoding DNA will share substantial sequence homology (greater than about 90%) with the α_7 DNAs described or deposited herein. DNA or RNA encoding a splice variant may share less than 90% overall sequence homology with the DNA or RNA provided herein, but such DNA would include regions of nearly 100% homology to the above-described DNA.

The α_7 subunits derived from the above-described DNA are expected to bind to the neurotoxin, α-bungarotoxin (α-bgtx). The activity of AChRs that contain α_7 subunits should be inhibited upon interaction with α-bgtx. Amino acid residues 210 through 217, as set forth in SEQ ID No. 8, are believed to be important

elements in the binding of α-bgtx (see, for example, Chargeaux et al. (1992) 13:299-301).

As used herein, a human beta subunit gene is a gene that encodes a beta subunit of a human neuronal nicotinic acetylcholine receptor. Assignment of the name "beta" to a putative nNAChR subunit, according to Deneris et al. supra, is based on the lack of adjacent cysteine residues (which are characteristic of alpha subunits). The beta subunit is frequently referred to as the structural NAChR subunit (although it is possible that beta subunits also have ACh binding properties). Combination of beta subunit(s) with appropriate alpha subunit(s) leads to the formation of a functional receptor. As used herein, a beta subunit refers to a nNAChR subunit that is encoded by DNA that hybridizes under high stringency conditions to at least one of the nNAChR-encoding DNAs (or deposited clones) disclosed herein. A beta subunit forms a functional NAChR, as assessed by methods described herein or known to those of skill in this art, with appropriate alpha subunit(s).

Also contemplated are beta subunits encoded by DNAs that encode beta subunits as defined above, but that by virtue of degeneracy of the genetic code do not necessarily hybridize to the disclosed DNA or deposited clones under the specified hybridization conditions. Such subunits also form functional receptors, as assessed by the methods described herein or known to those of skill in the art, in combination with appropriate alpha subunit(s). Typically, unless a beta subunit is encoded by RNA that arises as a splice variant, beta-encoding DNA and the beta subunit encoded thereby share substantial sequence homology with the beta-encoding DNA and beta subunit protein described herein. It is understood that DNA or RNA encoding a splice variant may share less than 90% overall homology with the DNA or RNA provided herein,

but such DNA will include regions of nearly 100% homology to the DNA described herein.

As used herein, "β_4 subunit DNA" refers to DNA encoding a neuronal nicotinic acetylcholine receptor subunit of the same name. Such DNA can be characterized in a number of ways, for example, the nucleotides of said DNA may encode the amino acid sequence set forth in SEQ. ID No. 12. Presently preferred β_4-encoding DNAs can be characterized as DNA which hybridizes under high stringency conditions to the coding sequence set forth in SEQ. ID No. 11 (preferably to substantially the entire coding sequence thereof, i.e., nucleotides 87-1583). Especially preferred β_4-encoding DNAs of the invention are characterized as having substantially the same nucleotide sequence as set forth in SEQ. ID No. 11.

Typically, unless a β_4 subunit arises as a splice variant, β_4-encoding DNA will share substantial sequence homology (greater than about 90%) with the β_4 DNAs described or deposited herein. DNA or RNA encoding a splice variant may share less than 90% overall sequence homology with the DNA or RNA provided herein, but such DNA would include regions of nearly 100% homology to the above-described DNA.

DNA encoding human neuronal nicotinic AChR alpha and beta subunits may be isolated by screening suitable human cDNA or human genomic libraries under suitable hybridization conditions with DNA disclosed herein (including nucleotides derived from any of SEQ ID Nos. 5, 7 or 11, or with any of the deposited clones referred to herein (e.g., ATCC accession no. 69239 or 69152). Suitable libraries can be prepared from neuronal tissue samples, hippocampus tissue, or cell lines, such as the human neuroblastoma cell line IMR32 (ATCC Accession No. CCL127), and the like. The library is

preferably screened with a portion of DNA including the entire subunit-encoding sequence thereof, or the library may be screened with a suitable probe.

As used herein, a probe is single-stranded DNA or RNA that has a sequence of nucleotides that includes at least 14 contiguous bases that are the same as (or the complement of) any 14 bases set forth in any of SEQ ID Nos. 1, 3, 5, 7, 9, or 11, or in the subunit encoding DNA in any of the deposited clones described herein (e.g., ATCC accession no. 69239 or 69152). Preferred regions from which to construct probes include 5' and/or 3' coding sequences, sequences predicted to encode transmembrane domains, sequences predicted to encode the cytoplasmic loop, signal sequences, acetylcholine (ACh) and α-bungarotoxin (α-bgtx) binding sites, and the like. Amino acids 210-220 are typically involved in ACh and α-bgtx binding. The approximate amino acid residues which are predicted to comprise such regions for other preferred probes are set forth in the following table:

Subunit	Signal Sequence	TMD1*	TMD2	TMD3	TMD4	Cytoplasmic Loop
α_2	1-55	264-289	297-320	326-350	444-515	351-443
α_3	1-30	240-265	273-296	302-326	459-480	327-458
α_4	1-33	241-269	275-289	303-330	593-618	594-617
α_7	1-23	229-256	262-284	290-317	462-487	318-461
β_2	1-25	234-259	267-288	295-320	453-477	321-452
β_4	1-23	234-258	264-285	290-319	454-478	320-453

* TMD = transmembrane domain

Alternatively, portions of the DNA can be used as primers to amplify selected fragments in a particular library.

After screening the library, positive clones are identified by detecting a hybridization signal; the identified clones are characterized by restriction enzyme mapping and/or DNA sequence analysis, and then examined, by comparison with the sequences set forth herein or with the deposited clones described herein, to ascertain whether they include DNA encoding a complete alpha or beta subunit. If the selected clones are incomplete, they may be used to rescreen the same or a different library to obtain overlapping clones. If desired, the library can be rescreened with positive clones until overlapping clones that encode an entire alpha or beta subunit are obtained. If the library is a cDNA library, then the overlapping clones will include an open reading frame. If the library is genomic, then the overlapping clones may include exons and introns. In both instances, complete clones may be identified by comparison with the DNA and encoded proteins provided herein.

Complementary DNA clones encoding various human nNAChR alpha and beta subunits have been isolated. Each subunit appears to be encoded by a different gene. The DNA clones provided herein may be used to isolate genomic clones encoding each subunit and to isolate any splice variants by screening libraries prepared from different neural tissues. Nucleic acid amplification techniques, which are well known in the art, can be used to locate splice variants of human NAChR subunits. This is accomplished by employing oligonucleotides based on DNA sequences surrounding divergent sequence(s) as primers for amplifying human RNA or genomic DNA. Size and sequence determinations of the amplification products can reveal the existence of splice variants. Furthermore, isolation of human genomic DNA sequences by hybridization

can yield DNA containing multiple exons, separated by introns, that correspond to different splice variants of transcripts encoding human NAChR subunits.

It has been found that not all subunits are expressed in all neural tissues or in all portions of the brain. Thus, in order to isolate cDNA encoding particular subunits or splice variants of such subunits, it is preferable to screen libraries prepared from different neuronal or neural tissues. Preferred libraries for obtaining DNA encoding each subunit include: hippocampus to isolate human α_4- and α_5-encoding DNA; IMR32 (human neuroblastoma cells; ATCC Accession No. CCL127) to isolate human α_3-, α_5-, α_7- and β_4-encoding DNA, thalamus to isolate α_2 and β_2-encoding DNA; and the like.

It appears that the distribution of expression of human neuronal nicotinic AChRs differs from the distribution of such receptors in rat. For example, RNA encoding the rat α_4 subunit is abundant in rat thalamus, but is not abundant in rat hippocampus (see, e.g., Wada et al. (1989) J. Comp. Neurol 284:314-335). No α_4-encoding clones could be obtained, however, from a human thalamus library. Instead, human α_4 clones were ultimately obtained from a human hippocampus library. Thus, the distribution of α_4 nNAChR subunit in humans and rats appears to be quite different.

Rat α_3 subunit appears to be a CNS-associated subunit that is abundantly expressed in the thalamus and weakly expressed in the brain stem (see, e.g., Boulter et al. (1986) Nature 319:368-374; Boulter et al. (1987) Proc. Natl. Acad. Sci. USA 84:7763-7767; and Wada et al. (1989) J. Comp. Neurol 284:314-335). In efforts to clone DNA encoding the human nicotinic AChR α_3 subunit, however, several human libraries, including a thalamus library, were unsuccessfully screened. Surprisingly, clones

encoding human α_3 subunit were ultimately obtained from a brain stem library and from IMR32 cells that reportedly express few, if any, functional nicotinic acetylcholine receptors (see, e.g., Gotti et al. ((1986) Biochem. Biophys. Res. Commun. 137: 1141-1147, and Clementi et al. (1986) J. Neurochem. 47: 291-297).

Rat α_7 subunit transcript reportedly is abundantly expressed in the hippocampus (see Seguela et al. (1993) J. Neurosci. 13:596-604). Efforts to clone DNA encoding a human α_7 subunit from a human hippocampus library ($1x10^6$ recombinants) were unsuccessful. Surprisingly, clones encoding a human NAChR α_7 subunit were ultimately obtained from an IMR32 cell cDNA library.

The above-described nucleotide sequences can be incorporated into vectors for further manipulation. As used herein, vector (or plasmid) refers to discrete elements that are used to introduce heterologous DNA into cells for either expression or replication thereof. Selection and use of such vehicles are well within the level of skill of the art.

As used herein, heterologous or foreign DNA and RNA are used interchangeably and refer to DNA or RNA that does not occur naturally as part of the genome of the cell in which it is present, or to DNA or RNA which is found in a location or locations in the genome that differ from that in which it occurs in nature. Typically, heterologous or foreign DNA and RNA refers to DNA or RNA that is not endogenous to the host cell and has been artificially introduced into the cell. Examples of heterologous DNA include DNA that encodes a human neuronal nicotinic AChR subunit, DNA that encodes RNA or proteins that mediate or alter expression of endogenous DNA by affecting transcription, translation, or other regulatable biochemical processes, and the like. The

cell that expresses heterologous DNA may contain DNA encoding the same or different expression products. Heterologous DNA need not be expressed and may be integrated into the host cell genome or maintained episomally.

An expression vector includes vectors capable of expressing DNAs that are operatively linked with regulatory sequences, such as promoter regions, that are capable of effecting expression of such DNA fragments. Thus, an expression vector refers to a recombinant DNA or RNA construct, such as a plasmid, a phage, recombinant virus or other vector that, upon introduction into an appropriate host cell, results in expression of the cloned nucleic acids. Appropriate expression vectors are well known to those of skill in the art and include those that are replicable in eukaryotic cells and/or prokaryotic cells and those that remain episomal or those which integrate into the host cell genome. Presently preferred plasmids for expression of invention AChR subunits in eukaryotic host cells, particularly mammalian cells, include cytomegalovirus (CMV) promoter-containing vectors such as pCMV, pcDNA1, and the like, as well as MMTV promoter-containing vectors, such as pMAMneo, and the like.

As used herein, a promoter region refers to a segment of DNA that controls transcription of DNA to which it is operatively linked. The promoter region includes specific sequences that are sufficient for RNA polymerase recognition, binding and transcription initiation. This portion of the promoter region is referred to as the promoter. In addition, the promoter region includes sequences that modulate this recognition, binding and transcription initiation activity of RNA polymerase. These sequences may be *cis* acting or may be responsive to *trans* acting factors. Promoters, depending

upon the nature of the regulation, may be constitutive or regulated. Exemplary promoters contemplated for use in the practice of the present invention include the SV40 early promoter, the cytomegalovirus (CMV) promoter, the mouse mammary tumor virus (MMTV) steroid-inducible promoter, Moloney murine leukemia virus (MMLV) promoter, and the like.

As used herein, the term "operatively linked" refers to the functional relationship of nucleic acids with regulatory and effector sequences of nucleotides, such as promoters, enhancers, transcriptional and translational stop sites, and other signal sequences. For example, operative linkage of nucleic acids to a promoter refers to the physical and functional relationship between the nucleic acids and the promoter such that the transcription of such nucleic acids is initiated from the promoter by an RNA polymerase that specifically recognizes, binds to and transcribes the nucleic acids. In order to optimize expression and/or *in vitro* transcription, it may be necessary to remove or alter 5' and/or 3' untranslated portions of the clones to remove extra, potential alternative translation initiation (i.e., start) codons or other sequences that interfere with or reduce expression, either at the level of transcription or translation. Alternatively, consensus ribosome binding sites (see, for example, Kozak (1991) J. Biol. Chem. 266:19867-19870) can be inserted immediately 5' of the start codon to enhance expression. Furthermore, for expression of NAChR subunits in amphibian oocytes, it may be desirable to surround the subunit coding sequence with *Xenopus* β-globin gene 5' and 3' untranslated sequences for optimum protein production. For example, NAChR subunit coding sequences can be incorporated into vector pSP64T [see Krieg and Melton in *Nucleic Acids Research* 12:7057-7070 (1984)], a modified form of pSP64 (available from Promega, Madison, WI). The

coding sequence is inserted between β-globin gene 5' and 3' untranslated sequences located downstream of the SP6 promoter. *In vitro* transcripts can then be generated from the resulting vector. The desirability of (or need for) such modification may be empirically determined.

Those of skill in the art recognize that a variety of promoters, enhancers, signal sequences, and the like can be employed to promote the expression of the cloned sequences described herein. In addition, it is readily recognized that the regulatory elements employed in a given construct need not be obtained from the same source. Indeed, the regulatory elements employed in a given construct can be obtained from different sources, such that various combinations of regulatory elements can be combined in a particular construct for expression.

As used herein, expression refers to the process by which polynucleic acids are transcribed into mRNA and translated into peptides, polypeptides, or proteins. If the polynucleic acid is derived from genomic DNA, expression may, if an appropriate eukaryotic host cell or organism is selected, include splicing of the mRNA.

Particularly preferred vectors for transfection of mammalian cells are the pSV2dhfr expression vectors, which contain the SV40 early promoter, mouse dhfr gene, SV40 polyadenylation and splice sites and sequences necessary for maintaining the vector in bacteria, cytomegalovirus (CMV) promoter-based vectors such as pCDNA1 (Invitrogen, San Diego, CA), and MMTV promoter-based vectors such as pMAMneo (Clontech, Palo Alto, CA), and modifications thereof.

Full-length DNAs encoding human neuronal NAChR subunits have been inserted into vector pCMV-T7, a pUC19-

based mammalian cell expression vector containing the CMV promoter/enhancer, SV40 splice/donor sites located immediately downstream of the promoter, a polylinker downstream of the splice/donor sites, followed by an SV40 polyadenylation signal. Placement of NAChR subunit DNA between the CMV promoter and SV40 polyadenylation signal provides for constitutive expression of the foreign DNA in a mammalian host cell transfected with the construct. For inducible expression of human NAChR subunit-encoding DNA in a mammalian cell, the DNA can be inserted into a plasmid such as pMSG. This plasmid contains the mouse mammary tumor virus (MMTV) promoter for steroid-inducible expression of operatively associated foreign DNA. If the host cell does not express endogenous glucocorticoid receptors required for uptake of glucocorticoids (i.e., inducers of the MMTV promoter) into the cell, it is necessary to additionally transfect the cell with DNA encoding the glucocorticoid receptor (ATCC accession no. 67200). Full-length human DNA clones encoding human α_3, α_4, α_7, β_2 and β_4 have also been subcloned into pIBI24 (International Biotechnologies, Inc., New Haven, CT) or pCMV-T7-2 for synthesis of *in vitro* transcripts.

In accordance with another embodiment of the present invention, there are provided cells containing the above-described polynucleic acids (i.e., DNA or mRNA). Such host cells as bacterial, yeast, amphibian and mammalian cells can be used for replicating DNA and producing nAChR subunit(s). Methods for constructing expression vectors, preparing *in vitro* transcripts, transfecting DNA into mammalian cells, injecting oocytes, and performing electrophysiological and other analyses for assessing receptor expression and function as described herein are also described in PCT Application Nos. PCT/US91/02311 (now published as WO 91/15602), PCT/US91/05625 (now published as WO 92/02639) and PCT/US92/11090 (now published as WO 93/13423), and in

co-pending U.S. Application Serial Nos. 07/504,455, 07/563,751 and 07/812,254. The subject matter of each of these applications is hereby incorporated by reference herein in its entirety.

Incorporation of cloned DNA into a suitable expression vector, transfection of eukaryotic cells with a plasmid vector or a combination of plasmid vectors, each encoding one or more distinct genes or with linear DNA, and selection of transfected cells are well known in the art (see, e.g., Sambrook et al. (1989) Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press). Heterologous DNA may be introduced into host cells by any method known to those of skill in the art, such as transfection with a vector encoding the heterologous DNA by $CaPO_4$ precipitation (see, e.g., Wigler et al. (1979) Proc. Natl. Acad. Sci. 76:1373-1376) or lipofectamine (GIBCO BRL #18324-012). Recombinant cells can then be cultured under conditions whereby the subunit(s) encoded by the DNA is (are) expressed. Preferred cells include mammalian cells (e.g., HEK 293, CHO, GH3 and Ltk$^-$ cells), yeast cells (e.g., methylotrophic yeast cells, such as *Pichia pastoris*), bacterial cells (e.g., *Escherichia coli*), and the like. Especially preferred cells are those which are also capable of expressing endogenous or heterologous voltage-dependent calcium channels (see, for example, PCT Application No. US92/06903; now published as WO 93/04083).

While the nucleic acids provided herein may be expressed in any eukaryotic cell, including yeast cells (such as, for example, *P. pastoris* (see U.S. Patent Nos. 4,882,279, 4,837,148, 4,929,555 and 4,855,231), *Saccharomyces cerevisiae*, *Candida tropicalis*, *Hansenula polymorpha*, and the like), mammalian expression systems, including commercially available systems and other such

systems known to those of skill in the art, for expression of nucleic acids encoding the human neuronal nicotinic AChR subunits provided herein are presently preferred. *Xenopus* oocytes are preferred for expression of RNA transcripts of the DNA.

In preferred embodiments, DNA is ligated into a vector, and introduced into suitable host cells to produce transformed cell lines that express a specific human nNAChR receptor subunit, or specific combinations of subunits. The resulting cell lines can then be produced in quantity for reproducible quantitative analysis of the effects of drugs on receptor function. In other embodiments, mRNA may be produced by *in vitro* transcription of DNA encoding each subunit. This mRNA, either from a single subunit clone or from a combination of clones, can then be injected into *Xenopus* oocytes where the mRNA directs the synthesis of the human receptor subunits, which then form functional receptors. Alternatively, the subunit-encoding nucleic acids can be directly injected into oocytes for expression of functional receptors. The transfected mammalian cells or injected oocytes may then be used in the methods of drug screening provided herein.

Cloned full-length DNA encoding any of the subunits of human neuronal nicotinic AChR may be introduced into a plasmid vector for expression in a eukaryotic cell. Such DNA may be genomic DNA or cDNA. Host cells may be transfected with one or a combination of plasmids, each of which encodes at least one human neuronal nicotinic AChR subunit.

Eukaryotic cells in which DNA or RNA may be introduced include any cells that are transfectable by such DNA or RNA or into which such DNA or RNA may be injected. Preferred cells are those that can be

transiently or stably transfected and also express the DNA and RNA. Presently most preferred cells are those that can form recombinant or heterologous human neuronal nicotinic AChRs comprising one or more subunits encoded by the heterologous DNA. Such cells may be identified empirically or selected from among those known to be readily transfected or injected.

Exemplary cells for introducing DNA include cells of mammalian origin (e.g., COS cells, mouse L cells, Chinese hamster ovary (CHO) cells, human embryonic kidney cells, African green monkey cells, GH3 cells and other such cells known to those of skill in the art), amphibian cells (e.g., *Xenopus laevis* oöcytes), yeast cells (e.g., *Saccharomyces cerevisiae*, *Pichia pastoris*), and the like. Exemplary cells for expressing injected RNA transcripts include *Xenopus laevis* oöcytes. Cells that are preferred for transfection of DNA are known to those of skill in the art or may be empirically identified, and include HEK 293 (which are available from ATCC under accession #CRL 1573); Ltk$^-$ cells (which are available from ATCC under accession #CCL1.3); COS-7 cells (which are available from ATCC under accession #CRL 1651); GH3 rat pituitary tumor cells (ATCC Accession No. CCL 82.1) and DG44 cells (dhfr$^-$ CHO cells; see, e.g., Urlaub et al. (1986) Cell. Molec. Genet. 12: 555). Presently preferred cells include DG44 cells, GH3 and HEK 293 cells, particularly HEK 293 cells that have been adapted for growth in suspension and that can be frozen in liquid nitrogen and then thawed and regrown. HEK 293 cells are described, for example, in U.S. Patent No. 5,024,939 to Gorman (see, also, Stillman et al. (1985) Mol. Cell. Biol. 5:2051-2060). Presently preferred cells also include those which are capable of expressing endogenous or heterologous voltage-dependent calcium channels.

Nucleic acids may be stably incorporated into cells or may be transiently introduced using methods known in the art. Stably transfected mammalian cells may be prepared by transfecting cells with an expression vector having a selectable marker gene (such as, for example, the gene for thymidine kinase, dihydrofolate reductase, neomycin resistance, and the like), and growing the transfected cells under conditions selective for cells expressing the marker gene. To produce such cells, the cells should be transfected with a sufficient concentration of subunit-encoding nucleic acids to form human neuronal nicotinic AChRs that contain the human subunits encoded by heterologous DNA. The precise amounts and ratios of DNA encoding the subunits may be empirically determined and optimized for a particular combination of subunits, cells and assay conditions. Recombinant cells that express neuronal nicotinic AChR containing subunits encoded only by the heterologous DNA or RNA are especially preferred.

Heterologous DNA may be maintained in the cell as an episomal element or may be integrated into chromosomal DNA of the cell. The resulting recombinant cells may then be cultured or subcultured (or passaged, in the case of mammalian cells) from such a culture or a subculture thereof. Methods for transfection, injection and culturing recombinant cells are known to the skilled artisan. Similarly, the human neuronal nicotinic AChR subunits may be purified using protein purification methods known to those of skill in the art. For example, antibodies or other ligands that specifically bind to one or more of the subunits may be used for affinity purification of the subunit or human neuronal nicotinic AChRs containing the subunits.

In accordance with yet another embodiment of the present invention, there are provided antibodies

generated against the above-described subunit proteins. Such antibodies can be employed for studying receptor tissue localization, subunit composition, structure of functional domains, as well as in diagnostic applications, therapeutic applications, and the like. Preferably, for therapeutic applications, the antibodies employed will be monoclonal antibodies.

The above-described antibodies can be prepared employing standard techniques, as are well known to those of skill in the art, using the invention subunit proteins or portions thereof as antigens for antibody production. Both anti-peptide and anti-fusion protein antibodies can be used [see, for example, Bahouth et al. (1991) Trends Pharmacol Sci. vol. 12:338-343; Current Protocols in Molecular Biology (Ausubel et al., eds.) John Wiley and Sons, New York (1989)]. Factors to consider in selecting portions of the NAChR subunits for use as immunogen (as either a synthetic peptide or a recombinantly produced bacterial fusion protein) include antigenicity, accessibility (i.e., extracellular and cytoplasmic domains), uniqueness to the particular subunit, etc.

The availability of subunit-specific antibodies makes possible the application of the technique of immunohistochemistry to monitor the distribution and expression density of various subunits (e.g., in normal vs diseased brain tissue). Such antibodies could also be employed for diagnostic and therapeutic applications.

In accordance with still another embodiment of the present invention, there are provided methods for modulating the ion channel activity of receptor(s) of the invention by contacting said receptor(s) with an effective amount of the above-described antibodies.

The antibodies of the invention can be administered to a subject employing standard methods, such as, for example, by intraperitoneal, intramuscular, intravenous, or subcutaneous injection, implant or transdermal modes of administration, and the like. One of skill in the art can readily determine dose forms, treatment regiments, etc, depending on the mode of administration employed.

In accordance with one embodiment of the present invention, methods for producing cells that express human neuronal nicotinic AChR subunits and functional receptors are also provided. In one such method, host cells are transfected with DNA encoding at least one alpha subunit of a neuronal nicotinic acetylcholine receptor and at least one beta subunit of a neuronal nicotinic acetylcholine receptor. Using methods such as northern blot or slot blot analysis, transfected cells that contain alpha and/or beta subunit encoding DNA or RNA can be selected. Transfected cells are also analyzed to identify those that express NAChR protein. Analysis can be carried out, for example, by measuring the ability of cells to bind acetylcholine, nicotine, or a nicotine agonist, compared to the nicotine binding ability of untransfected host cells or other suitable control cells, by electrophysiologically monitoring the currents through the cell membrane in response to a nicotine agonist, and the like.

In particularly preferred aspects, eukaryotic cells which contain heterologous DNAs express such DNA and form recombinant functional neuronal nicotinic AChR(s). In more preferred aspects, recombinant neuronal nicotinic AChR activity is readily detectable because it is a type that is absent from the untransfected host cell or is of a magnitude not exhibited in the untransfected cell. Such cells that contain recombinant receptors

could be prepared, for example, by causing cells transformed with DNA encoding the human neuronal nicotinic AChR α_3 and β_4 subunits to express the corresponding proteins. The resulting synthetic or recombinant receptors would contain only the α_3 and β_4 nNAChR subunits. Such receptors would be useful for a variety of applications, e.g., as part of an assay system free of the interferences frequently present in prior art assay systems employing non-human receptors or human tissue preparations. Furthermore, testing of single receptor subunits with a variety of potential agonists or antagonists would provide additional information with respect to the function and activity of the individual subunits. Such information is expected to lead to the identification of compounds which are capable of very specific interaction with one or more of the receptor subunits. Such specificity may prove of great value in medical application.

In another aspect, the invention comprises functional peptide fragments, and functional combinations thereof, encoded by the DNAs of the invention. Such functional peptide fragments can be produced by those skilled in the art, without undue experimentation, by eliminating some or all of the amino acids in the sequence not essential for the peptide to function as a NAChR. A determination of the amino acids that are essential for NAChR function is made, for example, by systematic digestion of the DNAs encoding the peptides and/or by the introduction of deletions into the DNAs. The modified (e.g., deleted or digested) DNAs are expressed, for example, by transcribing the DNA and then introducing the resulting mRNA into *Xenopus* oocytes, where translation of the mRNAs will occur. Functional analysis of the proteins thus expressed in the oocytes is accomplished by exposing the oocytes to ligands known to bind to and functionally activate NAChR, and then

monitoring the oocytes to see if endogenous channels are in turn activated. If currents are detected, the fragments are functional as NAChR.

Thus, DNA encoding one or more human neuronal nicotinic AChR subunits may be introduced into suitable host cells (e.g., eukaryotic or prokaryotic cells) for expression of individual subunits and functional NAChRs. Preferably combinations of alpha and beta subunits may be introduced into cells: such combinations include combinations of any one or more of α_1, α_2, α_3, α_4, α_5 and α_7 with β_2 or β_4. Sequence information for α_1 is presented in Biochem. Soc. Trans. (1989) 17:219-220; sequence information for α_5 is presented in Proc. Natl. Acad. Sci.USA (1992) 89:1572-1576; and sequence information for α_2, α_3, α_4, α_7, β_2 and β_4 is presented in the Sequence Listing provided herewith. Presently preferred combinations of subunits include any one or more of α_1, α_2, α_3 or α_4 with β_4; or α_2, α_3 or α_4 in combination with either β_2 or β_4. It is recognized that some of the subunits may have ion transport function in the absence of additional subunits. For example, the α_7 subunit may function in the absence of any added beta subunit.

As used herein, "α_2 subunit DNA" refers to DNA that encodes a human neuronal nicotinic acetylcholine receptor subunit of the same name, and to DNA that hybridizes under conditions of high stringency to the DNA of SEQ ID No. 1, or to the DNA of deposited clone having ATCC Accession No. 68277, or to DNA that encodes the amino acid sequence set forth in SEQ ID No. 2. Typically, unless an α_2 subunit arises as a splice variant, an α_2 DNA shares substantial sequence homology (greater than about 90%) with the α_2 DNA described herein. DNA or RNA encoding a splice variant may share less than 90% overall sequence homology with the DNA or RNA described herein, but such a splice variant would include

regions of nearly 100% homology to the above-described DNA.

As used herein, "α_3 subunit DNA" refers to DNA that encodes a neuronal subunit of the same name, and to DNA that hybridizes under conditions of high stringency to the DNA of SEQ ID No. 3, or to the DNA of deposited clone having ATCC Accession No. 68278, or to DNA that encodes the amino acid sequence set forth in SEQ ID No. 4. Typically, unless an α_3 arises as a splice variant, an α_3 DNA shares substantial sequence homology (greater than about 90%) with the α_3 DNA described herein. DNA or RNA encoding a splice variant may share less than 90% overall sequence homology with the DNA or RNA provided herein, but such a splice variant would include regions of nearly 100% homology to the above described DNA.

As used herein, "α_5 subunit DNA" refers to DNA that encodes a human neuronal nicotinic acetylcholine receptor subunit of the same name, as described, for example, by Chini et al. (1992) Proc. Natl. Acad. Sci. USA 89:1572-1576.

As used herein, "β_2 subunit DNA" refers to DNA that encodes a neuronal subunit of the same name and, to DNA that hybridizes under conditions of high stringency to the DNA of SEQ ID No. 9, or to the DNA of deposited clone HnAChRβ2, having ATCC Accession No. 68279, or to DNA encoding the amino acid sequence set forth in SEQ ID No. 10. Typically, unless a β_2 subunit arises as a splice variant, a β_2 DNA shares substantial sequence homology (greater than about 90%) with the β_2 DNA described herein. DNA or RNA encoding a splice variant may share overall less than 90% homology with the DNA or RNA provided herein, but such a splice variant would include regions of nearly 100% homology to the above-described DNA.

In certain embodiments, eukaryotic cells with heterologous human neuronal nicotinic AChRs are produced by introducing into the cell a first composition, which contains at least one RNA transcript that is translated in the cell into a subunit of a human neuronal nicotinic AChR. In preferred embodiments, the subunits that are translated include an alpha subunit of a human neuronal nicotinic AChR. More preferably, the composition that is introduced contains an RNA transcript which encodes an alpha subunit and also contains an RNA transcript which encodes a beta subunit of a human neuronal nicotinic AChR. RNA transcripts can be obtained from cells transfected with DNAs encoding human neuronal nicotinic acetylcholine receptor subunits or by *in vitro* transcription of subunit-encoding DNAs. Methods for *in vitro* transcription of cloned DNA and injection of the resulting mRNA into eukaryotic cells are well known in the art. Amphibian oocytes are particularly preferred for expression of *in vitro* transcripts of the human nNAChR DNA clones provided herein. See, for example, Dascal (1989) CRC Crit. Rev. Biochem. 22:317-387, for a review of the use of *Xenopus* oocytes to study ion channels.

Thus, pairwise (or stepwise) introduction of DNA or RNA encoding alpha and beta subunits into cells is possible. The resulting cells may be tested by the methods provided herein or known to those of skill in the art to detect functional AChR activity. Such testing will allow the identification of pairs of alpha and beta subunits that produce functional AChRs, as well as individual subunits that produce functional AChRs.

As used herein, a recombinant or heterologous human neuronal nicotinic AChR refers to a receptor that contains one or more subunits encoded by heterologous DNA that has been introduced into and expressed in cells

capable of expressing receptor protein. A recombinant human neuronal nicotinic AChR may also include subunits that are produced by DNA endogenous to the host cell. In certain embodiments, recombinant or heterologous human neuronal nicotinic AChR may contain only subunits that are encoded by heterologous DNA.

Recombinant receptors on recombinant eukaryotic cell surfaces may contain one or more subunits encoded by the DNA or mRNA encoding human neuronal nicotinic AChR subunits, or may contain a mixture of subunits encoded by the host cell and subunits encoded by heterologous DNA or mRNA. Recombinant receptors may be homogeneous or may be a mixture of subtypes. Mixtures of DNA or mRNA encoding receptors from various species, such as rats and humans, may also be introduced into the cells. Thus, a cell can be prepared that expresses recombinant receptors containing only α_3 and β_4 subunits, or any other combination of alpha and beta subunits provided herein. For example, α_4 and/or α_7 subunits of the present invention can be co-expressed with β_2 and/or β_4 receptor subunits; similarly, β_4 subunits according to the present invention can be co-expressed with α_2, α_3, α_4, α_5 and/or α_7 receptor subunits. As noted previously, some of the nNAChR subunits may be capable of forming functional receptors in the absence of other subunits, thus co-expression is not always required to produce functional receptors.

As used herein, activity of a human neuronal nicotinic AChR refers to any activity characteristic of an NAChR. Such activity can typically be measured by one or more *in vitro* methods, and frequently corresponds to an *in vivo* activity of a human neuronal nicotinic AChR. Such activity may be measured by any method known to those of skill in the art, such as, for example,

measuring the amount of current which flows through the recombinant channel in response to a stimulus.

Methods to determine the presence and/or activity of human neuronal nicotinic AChRs include assays that measure nicotine binding, ^{86}Rb ion-flux, Ca^{2+} influx, the electrophysiological response of cells, the electrophysiological response of oocytes transfected with RNA from the cells, and the like. In particular, methods are provided herein for the measurement or detection of an AChR-mediated response upon contact of cells containing the DNA or mRNA with a test compound.

As used herein, a functional neuronal nicotinic AChR is a receptor that exhibits an activity of neuronal nicotinic AChRs as assessed by any *in vitro* or *in vivo* assay disclosed herein or known to those of skill in the art. Possession of any such activity that may be assessed by any method known to those of skill in the art and provided herein is sufficient to designate a receptor as functional. Methods for detecting NAChR protein and/or activity include, for example, assays that measure nicotine binding, ^{86}Rb ion-flux, Ca^{2+} influx, the electrophysiological response of cells containing heterologous DNA or mRNA encoding one or more receptor subunits, and the like. Since all combinations of alpha and beta subunits may not form functional receptors, numerous combinations of alpha and beta subunits should be tested in order to fully characterize a particular subunit and cells which produce same. Thus, as used herein, "functional" with respect to a recombinant or heterologous human neuronal nicotinic AChR means that the receptor channel is able to provide for and regulate entry of human neuronal nicotinic AChR-permeable ions, such as, for example, Na^+, K^+, Ca^{2+} or Ba^{2+}, in response to a stimulus and/or bind ligands with affinity for the receptor. Preferably such human neuronal nicotinic AChR

activity is distinguishable, such as by electrophysiological, pharmacological and other means known to those of skill in the art, from any endogenous nicotinic AChR activity that may be produced by the host cell.

In accordance with a particular embodiment of the present invention, recombinant human neuronal nicotinic AChR-expressing mammalian cells or oocytes can be contacted with a test compound, and the modulating effect(s) thereof can then be evaluated by comparing the AChR-mediated response in the presence and absence of test compound, or by comparing the AChR-mediated response of test cells, or control cells (i.e., cells that do not express nNAChRs), to the presence of the compound.

As used herein, a compound or signal that "modulates the activity of a neuronal nicotinic AChR" refers to a compound or signal that alters the activity of NAChR so that activity of the NAChR is different in the presence of the compound or signal than in the absence of the compound or signal. In particular, such compounds or signals include agonists and antagonists. The term agonist refers to a substance or signal, such as ACh, that activates receptor function; and the term antagonist refers to a substance that interferes with receptor function. Typically, the effect of an antagonist is observed as a blocking of activation by an agonist. Antagonists include competitive and non-competitive antagonists. A competitive antagonist (or competitive blocker) interacts with or near the site specific for the agonist (e.g., ligand or neurotransmitter) for the same or closely situated site. A non-competitive antagonist or blocker inactivates the functioning of the receptor by interacting with a site other than the site that interacts with the agonist.

As understood by those of skill in the art, assay methods for identifying compounds that modulate human neuronal nicotinic AChR activity (e.g., agonists and antagonists) generally require comparison to a control. One type of a "control" cell or "control" culture is a cell or culture that is treated substantially the same as the cell or culture exposed to the test compound, except the control culture is not exposed to test compound. For example, in methods that use voltage clamp electrophysiological procedures, the same cell can be tested in the presence and absence of test compound, by merely changing the external solution bathing the cell. Another type of "control" cell or "control" culture may be a cell or a culture of cells which are identical to the transfected cells, except the cells employed for the control culture do not express functional human neuronal nicotinic AChRs. In this situation, the response of test cell to test compound is compared to the response (or lack of response) of receptor-negative (control) cell to test compound, when cells or cultures of each type of cell are exposed to substantially the same reaction conditions in the presence of compound being assayed.

The functional recombinant human neuronal nicotinic AChR includes at least an alpha subunit, or an alpha subunit and a beta subunit of a human neuronal nicotinic AChR. Eukaryotic cells expressing these subunits have been prepared by injection of RNA transcripts and by transfection of DNA. Such cells have exhibited nicotinic AChR activity attributable to human neuronal nicotinic AChRs that contain one or more of the heterologous human neuronal nicotinic AChR subunits. For example, *Xenopus laevis* oocytes that had been injected with *in vitro* transcripts of the DNA encoding human neuronal nicotinic AChR α_3 and β_4 subunits exhibited AChR agonist induced currents; whereas cells that had been

injected with transcripts of either the α_3 or β_4 subunit alone did not. In addition, HEK 293 cells that had been co-transfected with DNA encoding human neuronal NAChR α_3 and β_4 subunits exhibited AChR agonist-induced increases in intracellular calcium concentration, whereas control HEK 293 cells (i.e., cells that had not been transfected with α_3- and β_4-encoding DNA) did not exhibit any AChR agonist-induced increases in intracellular calcium concentration.

With respect to measurement of the activity of functional heterologous human neuronal nicotinic AChRs, endogenous AChR activity and, if desired, activity of AChRs that contain a mixture of endogenous host cell subunits and heterologous subunits, should, if possible, be inhibited to a significant extent by chemical, pharmacological and electrophysiological means.

Deposits

The deposited clones have been deposited at the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland, U.S.A. 20852, under the terms of the Budapest Treaty on the International Recognition of Deposits of Microorganisms for Purposes of Patent Procedure and the Regulations promulgated under this Treaty. Samples of the deposited material are and will be available to industrial property offices and other persons legally entitled to receive them under the terms of the Treaty and Regulations and otherwise in compliance with the patent laws and regulations of the United States of America and all other nations or international organizations in which this application, or an application claiming priority of this application, is filed or in which any patent granted on any such application is granted. In particular, upon issuance of a U.S. patent based on this or any application claiming

priority to or incorporating this application by reference thereto, all restrictions upon availability of the deposited material will be irrevocably removed.

The invention will now be described in greater detail with reference to the following non-limiting examples.

Example 1
Isolation of DNA Encoding Human nNAChR Subunits

A. DNA Encoding a Human nNAChR β_4 Subunit

Random primers were used in synthesizing cDNA from RNA isolated from the IMR32 human neuroblastoma cell line (the cells had been grown in 1mM dibutyryl cAMP for 10 days prior to constructing the library). The library constructed from the cDNAs was screened with a fragment of a rat nicotinic AChR β_4 subunit cDNA. Hybridization was performed at 42°C in 5X SSPE, 5X Denhardt's solution, 50% formamide, 200 μg/ml herring sperm DNA and 0.2% SDS. Washes were performed in 0.1X SSPE, 0.2% SDS at 65°C. Five clones were identified that hybridized to the probe.

The five clones were plaque-purified and characterized by restriction enzyme mapping and DNA sequence analysis. The insert DNA of one of the five clones contained the complete coding sequence of a β_4 subunit of a human nicotinic AChR (see nucleotides 87-1583 of SEQ ID No. 11). The amino acid sequence deduced from the nucleotide sequence of the full-length clone has ~82% identity with the amino acid sequence deduced from the rat nicotinic AChR β_4 subunit DNA. Several regions of the deduced rat and human β_4 amino acid sequences are notably dissimilar: amino acids 1-23 (the human sequence has only ~36% identity with respect to the rat sequence), 352-416 (the human sequence has only ~48% identity with

respect to the rat sequence), and 417-492 (the human sequence has only ~78% identity with respect to the rat sequence). Furthermore, amino acids 376-379 in the rat β_4 subunit are not contained in the human β_4 subunit.

B. DNA Encoding a Human nNAChR α_7 Subunit

An amplified IMR32 cell cDNA library (1×10^6 recombinants; cells treated for 10 days with 1mM dibutyryl cAMP prior to library construction) was screened with a fragment of a rat nicotinic AChR α_7 subunit cDNA. The hybridization conditions were identical to those described above for screening an IMR32 cell cDNA library with the rat β_4 subunit DNA. Washes were performed in 0.2X SSPE, 0.2% SDS at 65°C. Seven positive clones were identified by hybridization to the labeled rat DNA probe. Six of the clones were plaque-purified and characterized by restriction enzyme mapping and DNA sequence analysis. One of the clones contains the complete coding sequence of a human AChR receptor α_7 subunit gene (see nucleotides 73-1581 of SEQ ID No. 7).

C. DNA Encoding a Human nNAChR α_4 Subunit

Random primers were used in synthesizing cDNA from RNA isolated from human hippocampus tissue. cDNAs larger than 2.0 kb were inserted into the λgt10 phage vector to create a cDNA library. Approximately 1×10^6 recombinants were screened with a fragment of a DNA encoding a rat nicotinic AChR α_4 subunit using the same hybridization and washing conditions as described above for screening an IMR32 cell cDNA library for α_7 subunit cDNAs. Three clones hybridized strongly to the probe. Two of these three clones, designated KEα4.1 and KEα4.2, have been deposited with the American Type Culture Collection (ATCC, Rockville, MD) and assigned accession nos. 69152 and 69239, respectively.

Characterization of the plaque-purified clones revealed that one of the clones, KEα4.2, contains the complete coding sequence of a human nicotinic AChR α4 subunit gene (coding sequence of this human α_4 subunit cDNA is provided as nucleotides 173-2056 in SEQ ID No. 5). Comparison of the 5' ends of the coding sequences of the human and rat α4 subunit cDNAs reveals, among other differences, that the rat sequence contains an 18-nucleotide segment that is not present in the human sequence.

D. DNA Encoding Human nNAChR α_2, α_3, & β_2 Subunits

Plasmids containing DNA that encodes and/or that can be used to isolate nucleic acids that encode human neuronal nicotinic acetylcholine receptor α_2, α_3 and β_2 subunits have been deposited with the American Type Culture Collection (ATCC). The clone names and deposit accession numbers are:

Subunit	Clone Name	ATCC Accession No.
α_2	HnAChRα2	68277
α_3	HnACHRα3	68278
β_2	HnAChRβ2	68279

In addition, DNA sequences that encode full-length α_2, α_3 and β_2 subunits are set forth in SEQ ID Nos. 1, 3 and 9, respectively.

Example 2

Preparation of Constructs for the Expression of Recombinant Human Neuronal Nicotinic AChR Subunits

Isolated cDNAs encoding human neuronal nicotinic AChR subunits were incorporated into vectors for use in expressing the subunits in mammalian host cells and for use in generating *in vitro* transcripts to be expressed in *Xenopus* oocytes. Several different vectors were utilized in preparing the constructs as follows.

A. Construct for Expression of a Human nNAChR α_3 Subunit

DNA encoding a human neuronal nicotinic AChR α_3 subunit was subcloned into the pCMV-T7-2 general expression vector to create pCMV-KEα3. Plasmid pCMV-T7-2 (see Figure 1) is a pUC19-based vector that contains a CMV promoter/enhancer, SV40 splice donor/splice acceptor sites located immediately downstream of the promoter, a T7 bacteriophage RNA polymerase promoter positioned downstream of the SV40 splice sites, an SV40 polyadenylation signal downstream of the T7 promoter, and a polylinker between the T7 promoter and the polyadenylation signal. This vector thus contains all the regulatory elements required for expression of heterologous DNA in a mammalian host cell, wherein the heterologous DNA has been incorporated into the vector at the polylinker. In addition, because the T7 promoter is located just upstream of the polylinker, this plasmid can be used for synthesis of *in vitro* transcripts from heterologous DNA that has been subcloned into the vector at the polylinker. Figure 1 also shows a restriction map of pCMV-T7-3. This plasmid is identical to pCMV-T7-2 except that the restriction sites in the polylinker are

in the opposite order as compared to the order in which they occur in pCMV-T7-2.

A 1.7 kb *Sfi*I (blunt-ended)/*Eco*RI DNA fragment containing nucleotides 27-1757 of SEQ ID No. 3 (i.e., the entire α_3 subunit coding sequence plus 12 nucleotides of 5' untranslated sequence and 204 nucleotides of 3' untranslated sequence) was ligated to *Eco*RV/*Eco*RI-digested pCMV-T7-2 to generate pCMV-KEα3. Plasmid pCMV-KEα3 was used for expression of the α_3 subunit in mammalian cells and for generating *in vitro* transcripts from the α_3 subunit DNA.

B. Constructs for Expression of a Human nNAChR β_4 Subunit

A 1.9 kb *Eco*RI DNA fragment containing nucleotides 1-1915 of SEQ ID No. 11 (i.e., the entire β_4 subunit coding sequence plus 86 nucleotides of 5' untranslated sequence and 332 nucleotides of 3' untranslated sequence) was ligated to *Eco*RI-digested pGEM7Zf(+) (Promega Catalog #P2251; Madison, WI). The resulting construct, KEβ4.6/pGEM, contains the T7 bacteriophage RNA polymerase promoter in operative association with two tandem β_4 subunit DNA inserts (in the same orientation) and was used in generating *in vitro* transcripts from the DNA.

The same 1.9 kb *Eco*RI DNA fragment containing nucleotides 1-1915 of SEQ ID No. 11 was ligated as a single insert to *Eco*RI-digested pCMV-T7-3 to generate pCMV-KEβ4. Plasmid pCMV-KEβ4 was used for expression of the β_4 subunit in mammalian cells and for generating *in vitro* transcripts of the β_4 subunit DNA.

C. Constructs for Expression of a Human nNAChR α_7 Subunit

Two pCMV-T7-based constructs were prepared for use in recombinant expression of a human neuronal nicotinic AChR α_7 subunit. The first construct, pCMV-KEα7.3, was prepared by ligating a 1.9 kb *Xho*I DNA fragment containing nucleotides 1-1876 of SEQ ID No. 7 (i.e., the entire α_7 subunit coding sequence plus 72 nucleotides of 5' untranslated sequence and 295 nucleotides of 3' untranslated sequence) to *Sal*I-digested pCMV-T7-3. The second construct, pCMV-KEα7, was prepared by replacing the 5' untranslated sequence of the 1.9 kb *Xho*I α_7 subunit DNA fragment described above with a consensus ribosome binding site (5'-GCCACC-3'; see Kozak (1987) *Nucl. Acids Res. 15*:8125-8148). The resulting modified fragment was ligated as a 1.8 kb *Bgl*II/*Xho*I fragment with *Bgl*II/*Sal*I-digested pCMV-T7-2 to generate pCMV-KEα7. Thus, in pCMV-KEα7, the translation initiation codon of the coding sequence of the α_7 subunit cDNA is preceded immediately by a consensus ribosome binding site.

D. Constructs for Expression of a Human nNAChR β_2 Subunit

DNA fragments encoding portions of a human neuronal nicotinic AChR β_2 subunit were ligated together to generate a full-length β_2 subunit coding sequence contained in plasmid pIBI24 (International Biotechnologies, Inc. (IBI), New Haven, CT). The resulting construct, Hβ2.1F, contains nucleotides 1-2448 of SEQ ID No. 9 (i.e., the entire β_2 subunit coding sequence, plus 264 nucleotides of 5' untranslated sequence and 675 nucleotides of 3' untranslated sequence) in operative association with the T7 promoter.

Because the 5' untranslated sequence of the β_2 subunit DNA contains a potential alternative translation initiation codon (ATG) beginning 11 nucleotides upstream (nucleotides 254-256 in SEQ ID No. 9) of the correct translation initiation codon (nucleotides 265-267 in SEQ ID No. 9), and because the use of the upstream ATG sequence to initiate translation of the β_2 DNA might result in the generation of an inoperative peptide (because the upstream ATG is not in the correct reading frame), an additional β_2-encoding construct was prepared as follows. A 2.2 kb *Ksp*I (blunt ended)/*Eco*RI DNA fragment containing nucleotides 260-2448 of SEQ ID No. 9 was ligated to *Not*I (blunt ended)/*Eco*RI-digested pCMV-T7-3 in operative association with the T7 promoter of the plasmid to create pCMV-KEβ2. The β_2 subunit DNA contained in pCMV-KEβ2 retains only 5 nucleotides of 5' untranslated sequence upstream of the correct translation initiation codon.

DNA encoding a human NAChR β2 subunit was also incorporated into expression vector pSP64T. Vector pSP64T [see Krieg and Melton in *Nucleic Acids Research* 12:7057-7070 (1984)] is a modified form of vector pSP64 (Promega). The human NAChR β2 subunit coding sequence (preceded by the consensus ribosome binding site), plus 405 nucleotides of 3' untranslated region, were incorporated into pSP64T at a unique restriction enzyme cloning site that is flanked by 5' and 3' untranslated sequences from the *Xenopus* β-globin gene. These sequences are located downstream of the SP6 promoter contained in pSP64T. The resulting vector, pSP64T-KEβ2RBS1, contains the human β2 subunit coding sequence in operable association with SP6 transcription regulatory regions for the production of *in vitro* transcripts of the heterologous DNA using the MEGAscript SP6 kit (Ambion, Catalog No. 1330).

E. Constructs for Expression of a Human nNAChR α_4 Subunit

A portion of the insert of clone KEα4.2 (see Example 1C), containing a human nNAChR α_4 subunit coding sequence, was incorporated into a modified vector pIBI24 as follows. Vector pIBI24 was modified by inserting a consensus ribosome binding site into the polylinker just upstream of an *Not*I site. The vector was digested with *Hin*dIII and *Nco*I. An *Nco*I-*Hin*dIII fragment containing a human nNAChR α_4 subunit coding sequence was obtained by digestion of a human nNAChR α_4 subunit cDNA-containing plasmid with *Hin*dIII (which cuts in a polylinker immediately 3' to the 3'untranslated sequence of KEα4.2 (see SEQ ID NO:5), followed by partial digestion with *Nco*I (to maintain an internal *Nco*I site, i.e., position 1956, SEQ ID NO:5) to cut at the junction of the translation initiation codon and 5' untranslated sequence of the α_4 subunit-encoding cDNA. The resulting 3.25 kb fragment was ligated with the *Hin*dIII-*Nco*I fragment of the modified pIBI24 vector to create pIBI-KEα4RBSf. Thus, pIBI-KEα4RBSf contains a consensus ribosome binding site followed immediately by the human nNAChR α_4 subunit coding sequence (nucleotides 173-2056 of SEQ ID NO:5) and ~1400 nucleotides of 3' untranslated sequence (including nucleotides 2057 - 2363 of SEQ ID NO:5). Because this construct contains a T7 promoter upstream of the α_4 subunit coding sequence, it can be used in generating *in vitro* transcripts from α_4 DNA.

Example 3

Expression of Recombinant Human Nicotinic AChR in Oocytes

Xenopus oocytes were injected with *in vitro* transcripts prepared from constructs containing DNA encoding α_3, α_7, β_2 and β_4 subunits. Electrophysiological measurements of the oocyte transmembrane currents were made using the two-electrode voltage clamp technique (see, e.g., Stuhmer (1992) *Meth. Enzymol. 207*:319-339).

1. Preparation of *in vitro* transcripts

Recombinant capped transcripts of pCMV-KEα3, pCMV-KEβ2, KEβ4.6/pGEM and pCMV-KEβ4 were synthesized from linearized plasmids using the mCAP RNA Capping Kit (Cat. #200350 from Stratagene, Inc., La Jolla, CA). Recombinant capped transcripts of pCMV-KEα7, pCMV-KEα7.3, pIBI-KEα4RBSf and Hβ2.1F were synthesized from linearized plasmids using the MEGAscript T7 *in vitro* transcription kit according to the capped transcript protocol provided by the manufacturer (Catalog #1334 from AMBION, Inc., Austin, TX). The mass of each synthesized transcript was determined by UV absorbance and the integrity of each transcript was determined by electrophoresis through an agarose gel.

2. Electrophysiology

Xenopus oocytes were injected with either 12.5, 50 or 125 ng of human nicotinic AChR subunit transcript per oocyte. The preparation and injection of oocytes were carried out as described by Dascal (1987) in *Crit. Rev. Biochem. 22*:317-387. Two-to-six days following mRNA injection, the oocytes were examined using the two-electrode voltage clamp technique. The cells were bathed in Ringer's solution (115 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$, 10 mM HEPES, pH 7.3) containing 1 μM atropine with

or without 100 μM d-tubocurarine. Cells were voltage-clamped at -60 to -80 mV. Data were acquired with Axotape software at 2-5 Hz. The agonists acetylcholine (ACh), nicotine, and cytisine were added at concentrations ranging from 0.1 μM to 100 μM. The results of electrophysiological analyses of the oocytes are summarized in Table 1.

Table 1

Template, ng RNA injected	Number of oocytes responding	Agonists	Current Amplitude
pCMV-KEα3, 12.5 ng	0 of 8	ACh, Nicotine	
KEβ4.6/pGEM, 12.5 ng	0 of 9	ACh, Nicotine	
pCMV-KEα3, 12.5 ng + KEβ4.6/pGEM, 12.5 ng	4 of 5	ACh, Nicotine	20-550 nA
pCMV-KEα3, 12.5 ng + KEβ4.6/pGEM, 12.5 ng	3 of 4	ACh, Cytisine, Nicotine	20-300 nA
pCMV-KEα3, 125 ng + and pCMV-KEβ4, 125 ng	5 of 5	ACh, Nicotine, Cytisine	200-500 nA
pCMV-KEα3, 125 ng + pCMV-KEβ4, 125 ng	6 of 6	ACh, Nicotine, Cytisine	100-400 nA
pCMV-KEα7.3, 125 ng	3 of 15	ACh	~20 nA
pCMV-KEα7, 125 ng	11 of 11	ACh	20-250 nA
pCMV-KEα3, 12.5 ng + pCMV-KEβ2, 12.5 ng	2 of 9	ACh, Nicotine	<10 nA
pCMV-KEα3, 125 ng + pCMV-KEβ2, 125 ng	0 of 9	ACh, Nicotine	
pCMV-KEα3, 125 ng + Hβ2.1F, 125 ng	13 of 16	ACh (100 μM) ACh (300 μM)	~20 nA ~80 nA
pIBI-KEα4RBSf, 125 ng + pCMV-KEβ4, 125 ng	3 of 3	ACh (30 μM)	~40 nA

a. Oocytes Injected with α_3, α_4 and/or β_4 Transcripts

Oocytes that had been injected with 12.5 ng of the α_3 transcript or 12.5 ng of the β_4 transcript did not

respond to application of up to 100 μM ACh, nicotine or cytisine. Thus, it appears that these subunits do not form functional homomeric nicotinic AChR channels. By contrast, oocytes injected with 12.5 or 125 ng of the α_3 transcript and 12.5 ng or 125 ng of the β_4 transcript exhibited detectable inward currents in response to ACh, nicotine, and cytisine at the tested concentrations (0.1 μM to 10 μM). Some differences in the kinetics of the responses to cytisine compared to nicotine and ACh were observed. The relative potency of the agonists appeared to be cytisine > ACh > nicotine, which differs from the results of similar studies of oocytes injected with transcripts of the rat nicotinic AChR α_3 and β_4 subunits (see, for example, Luetje *et al.* (1991) *J. Neurosci. 11*:837-845).

The responses to ACh and nicotine were reproducibly blocked by d-tubocurarine. For example, complete blockage of the response to ACh was observed in the presence of 100 μM d-tubocurarine. The inhibition appeared to be reversible. The responses to ACh, nicotine and cytisine were also at least partially blocked by 100 nM mecamylamine.

The current response of α_3-β_4-injected oocytes to 10 μM ACh was also examined in terms of membrane voltage. In these experiments, voltage steps were applied to the cells in the presence of ACh. The graph of current vs. voltage appeared typical of responses observed for Na^+, K^+-permeable channels. For example, the zero current level (reversal potential) is less than -40 mV. The contribution of Ca^{++} flux to the total current can be ascertained by varying the calcium concentration in the external medium and taking multiple current measurements at different holding potentials around the reversal potential. Such studies indicate that the channel carrying the current generated in

response to ACh treatment of α_3-β_4-injected oocytes is permeable to Na^+, K^+ and Ca^{++}.

As shown in Table 1, oocytes injected with 125 ng of the α_4 transcript and 125 ng of the β_4 transcript also exhibited detectable inward currents in response to acetylcholine.

b. Oocytes injected with α_7 subunit transcripts

As described in Example 2, two constructs were prepared for use in expressing the human neuronal nicotinic AChR α_7 subunit. Plasmid pCMV-KEα7.3 contains the α_7 subunit coding sequence with 72 nucleotides of 5' untranslated sequence upstream of the translation initiation codon. Plasmid pCMV-KEα7 contains the α_7 subunit coding sequence devoid of any 5' untranslated sequence and further contains a consensus ribosome binding site immediately upstream of the coding sequence.

Oocytes injected with 125 ng of α_7 transcript synthesized from pCMV-KEα7 displayed inward currents in response to 10 or 100 μM ACh. This response was blocked by 100 μM d-tubocurarine.

Oocytes injected with 125 ng of α_7 transcript synthesized from pCMV-KEα7.3 exhibited ACh-induced currents that were substantially weaker than those of oocytes injected with α_7 transcript synthesized from pCMV-KEα7. These results indicate that human neuronal nicotinic AChR α_7 subunit transcripts generated from α_7 subunit DNA containing a ribosome binding site in place of 5' untranslated sequence may be preferable for expression of the α_7 receptor in oocytes.

c. Oocytes injected with α_3 and β_2 subunit transcripts

As described in Example 2, two constructs were prepared for use in expressing the human neuronal nicotinic AChR β_2 subunit. Plasmid Hβ2.1F contains the β_2 subunit coding sequence with 266 nucleotides of 5' untranslated sequence upstream of the translation initiation codon. Plasmid pCMV-KEβ2 contains the β_2 subunit coding sequence and only 5 nucleotides of 5' untranslated sequence upstream of the translation initiation codon.

Oocytes injected with transcripts of pCMV-KEα3 and pCMV-KEβ2 displayed substantially no current in response to nicotinic AChR agonists. In contrast, oocytes injected with transcripts of pCMV-KEα3 and Hβ2.1F displayed ~20 nA inward currents in response to 100 μM ACh and ~80 nA inward currents in response to 300 μM ACh. The current response was blocked by 100 μM d-tubocurarine.

Example 4
Recombinant Expression of Human nNAChR Subunits in Mammalian Cells

Human embryonic kidney (HEK) 293 cells were transiently and stably transfected with DNA encoding human neuronal nicotinic AChR α_3 and β_4, or α_7 subunits. Transient transfectants were analyzed for expression of nicotinic AChR using various assays, e.g., electrophysiological methods, Ca^{2+}-sensitive fluorescent indicator-based assays and [^{125}I]-α-bungarotoxin-binding assays.

1. Transient Transfection of HEK Cells

Two transient transfections were performed. In one transfection, HEK cells were transiently co-transfected with DNA encoding α_3 (plasmid pCMV-KEα3) and β_4 (plasmid pCMV-KEβ4) subunits. In the other transfection, HEK cells were transiently transfected with DNA encoding the α_7 subunit (plasmid pCMV-KEα7). In both transfections, ~2 x 10^6 HEK cells were transiently transfected with 18 μg of the indicated plasmid(s) according to standard $CaPO_4$ transfection procedures [Wigler *et al.* (1979) *Proc. Natl. Acad. Sci. USA* 76:1373-1376]. In addition, 2 μg of plasmid pCMVβgal (Clontech Laboratories, Palo Alto, CA), which contains the *Escherichia coli* β-galactosidase gene fused to the CMV promoter, were co-transfected as a reporter gene for monitoring the efficiency of transfection. The transfectants were analyzed for β-galactosidase expression by measurement of β-galactosidase activity [Miller (1972) Experiments in Molecular Genetics, pp.352-355, Cold Spring Harbor Press]. Transfectants can also be analyzed for β-galactosidase expression by direct staining of the product of a reaction involving β-galactosidase and the X-gal substrate [Jones (1986) *EMBO 5*:3133-3142].

The efficiency of transfection of HEK cells with pCMV-KEα3/pCMV-KEβ4 was typical of standard efficiencies, whereas the efficiency of transfection of HEK cells with pCMV-KEα7 was below standard levels.

2. Stable Transfection of HEK Cells

HEK cells were transfected using the calcium phosphate transfection procedure [*Current Protocols in Molecular Biology, Vol. 1*, Wiley Inter-Science, Supplement 14, Unit 9.1.1-9.1.9 (1990)]. Ten-cm plates,

each containing one-to-two million HEK cells were transfected with 1 ml of DNA/calcium phosphate precipitate containing 9.5 μg pCMV-KEα3, 9.5 μg pCMV-KEβ4 and 1 μg pSV2neo (as a selectable marker). After 14 days of growth in media containing 1 μg/ml G418, colonies had formed and were individually isolated by using cloning cylinders. The isolates were subjected to limiting dilution and screened to identify those that expressed the highest level of nicotinic AChR, as described below.

3. Analysis of Transfectants

a. Fluorescent indicator-based assays

Activation of the ligand-gated nicotinic AChR by agonists leads to an influx of cations, including Ca^{++}, through the receptor channel. Ca^{++} entry into the cell through the channel can induce release of calcium contained in intracellular stores. Monovalent cation entry into the cell through the channel can also result in an increase in cytoplasmic Ca^{++} levels through depolarization of the membrane and subsequent activation of voltage-dependent calcium channels. Therefore, methods of detecting transient increases in intracellular calcium concentration can be applied to the analysis of functional nicotinic AChR expression. One method for measuring intracellular calcium levels relies on calcium-sensitive fluorescent indicators.

Calcium-sensitive indicators, such as fluo-3 (Catalog No. F-1241, Molecular Probes, Inc., Eugene, OR), are available as acetoxymethyl esters which are membrane permeable. When the acetoxymethyl ester form of the indicator enters a cell, the ester group is removed by cytosolic esterases, thereby trapping the free indicator in the cytosol. Interaction of the free indicator with calcium results in increased fluorescence of the

indicator; therefore, an increase in the intracellular Ca^{2+} concentration of cells containing the indicator can be expressed directly as an increase in fluorescence. An automated fluorescence detection system for assaying nicotinic AChR has been described in commonly assigned pending US Patent Application No. 07/812,254 and corresponding PCT Patent Application No. US92/11090.

HEK cells that were transiently or stably co-transfected with DNA encoding $\alpha 3$ and $\beta 4$ subunits were analyzed for expression of functional recombinant nicotinic AChR using the automated fluorescent indicator-based assay. The assay procedure was as follows.

Untransfected HEK cells (or HEK cells transfected with pCMV-T7-2) and HEK cells that had been co-transfected with pCMV-KE$\alpha 3$ and pCMV-KE$\beta 4$ were plated in the wells of a 96-well microtiter dish and loaded with fluo-3 by incubation for 2 hours at 20°C in a medium containing 20 μM fluo-3, 0.2% Pluronic F-127 in HBS (125 mM NaCl, 5 mM KCl, 1.8 mM $CaCl_2$, 0.62 mM $MgSO_4$, 6 mM glucose, 20 mM HEPES, pH 7.4). The cells were then washed with assay buffer (i.e., HBS). The antagonist d-tubocurarine was added to some of the wells at a final concentration of 10 μM. The microtiter dish was then placed into a fluorescence plate reader and the basal fluorescence of each well was measured and recorded before addition of 200 μM nicotine to the wells. The fluorescence of the wells was monitored repeatedly during a period of approximately 60 seconds following addition of nicotine.

The fluorescence of the untransfected HEK cells (or HEK cells transfected with pCMV-T7-2) did not change after addition of nicotine. In contrast, the fluorescence of the co-transfected cells, in the absence of d-tubocurarine, increased dramatically after addition

of nicotine to the wells. This nicotine-stimulated increase in fluorescence was not observed in co-transfected cells that had been exposed to the antagonist d-tubocurarine. These results demonstrate that the co-transfected cells express functional recombinant AChR that are activated by nicotine and blocked by d-tubocurarine.

b. α-Bungarotoxin binding assays

HEK293 cells transiently transfected with pCMV-KEα7 were analyzed for [^{125}I]-α-bungarotoxin (BgTx) binding. Both whole transfected cells and membranes prepared from transfected cells were examined in these assays. Rat brain membranes were included in the assays as a positive control.

Rat brain membranes were prepared according to the method of Hampson et al. (1987) J. Neurochem 49:1209. Membranes were prepared from the HEK cells transfected with pCMV-KEα7 and HEK cells transiently transfected with plasmid pUC19 only (negative control) according to the method of Perez-Reyes et al. (1989) Nature 340:233. Whole transfected and negative control cells were obtained by spraying the tissue culture plates with phosphate-buffered saline containing 0.1% (w/v) BSA. The cells were then centrifuged at low speed, washed once, resuspended in assay buffer (118 mM NaCl, 4.8 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 20 mM HEPES, 0.1% (w/v)BSA, 0.05% (w/v) bacitracin and 0.5 mM PMSF, pH 7.5) and counted.

Specific binding of [^{125}I]-α-BgTx to rat brain membranes was determined essentially as described by Marks et al. (1982) Molec. Pharmacol. 22:554-564, with several modifications. The membranes were washed twice in assay buffer. The assay was carried out in 12 x 75 mm

polypropylene test tubes in a total volume of 0.5 ml assay buffer. The membranes were incubated with 10 nM [^{125}I]-α-BgTx (New England Nuclear, Boston, MA) for one hour at 37°C. The assay mixtures were then centrifuged at 2300 x g for 10 minutes at 4°C. The supernatant was decanted and the pellets were washed twice with 2 ml aliquots of ice-cold assay buffer. The supernatants were decanted again and the radioactivity of the pellets was measured in a γ-counter. Non-specific binding was determined in the presence of 1 μM unlabeled α-BgTx. Specific binding was determined by subtracting nonspecific binding from total binding. Specific binding of [^{125}I]-α-BgTx to membranes prepared from transfected and negative control cells was determined as described for determining specific binding to rat brain membranes except that the assay buffer did not contain BSA, bacitracin and PMSF. Specific binding of [^{125}I]-α-BgTx to transfected and negative control whole cells was determined basically as described for determining specific binding to rat brain membranes.

[^{125}I]-α-BgTx binding was evaluated as a function of membrane concentration and as a function of incubation time. [^{125}I]-α-BgTx binding to rat brain membranes increased in a linear fashion with increasing amounts of membrane (ranging between 25-500 μg). The overall signal-to-noise ratio of binding (i.e., ratio of total binding to non-specific binding) was 3:1. Although some binding of [^{125}I]-α-BgTx to transfected cell membranes was detected, it was mostly non-specific binding and did not increase with increasing amounts of membrane. [^{125}I]-α-BgTx binding to the transfectants and negative control cells appeared to be similar.

To monitor [^{125}I]-α-BgTx binding to rat brain membranes and whole transfected and negative control cells, 300 μg of membrane or 500,000 cells were incubated

with 1 nM or 10 nM [^{125}I]-α-BgTx, respectively, at 37°C for various times ranging from 0-350 min. Aliquots of assay mixture were transferred to 1.5 ml microfuge tubes at various times and centrifuged. The pellets were washed twice with assay buffer. [^{125}I]-α-BgTx binding to rat brain membranes increased with time and was maximal after three hours. The binding profiles of the transfected and negative control cells were the same and differed from that of rat brain membranes.

Example 5

Characterization of Cell Lines Expressing nNAChRs

Recombinant cell lines generated by transfection with DNA encoding human neuronal nicotinic AChRs, such as those described in Example 3, can be further characterized using one or more of the following methods.

A. Northern or slot blot analysis for expression of α- and/or β-subunit encoding messages

Total RNA is isolated from ~1X10^7 cells and 10-15 μg of RNA from each cell type is used for northern or slot blot hybridization analysis. The inserts from human neuronal NAChR-encoding plasmids can be nick-translated and used as probe. In addition, the β-actin gene sequence (Cleveland et al. (1980) Cell 20:95-105) can be nick-translated and used as a control probe on duplicate filters to confirm the presence or absence of RNA on each blot and to provide a rough standard for use in quantitating differences in α- or β-specific mRNA levels between cell lines. Typical northern and slot blot hybridization and wash conditions are as follows:

hybridization in 5x SSPE, 5X Denhardt's solution, 50% formamide, at 42°C followed by washing in 0.2x SSPE, 0.1% SDS, at 65°C.

B. Nicotine-binding assay

Cell lines generated by transfection with human neuronal nicotinic AChR α- or α- and β-subunit-encoding DNA can be analyzed for their ability to bind nicotine, for example, as compared to control cell lines: neuronally-derived cell lines PC12 (Boulter et al., (1986), supra; ATCC #CRL1721) and IMR32 (Clementi, et al. (1986); Int. J. Neurochem. 47:291-297; ATCC #CCL127), and muscle-derived cell line BC3H1 (Patrick, et al., (1977); J. Biol. Chem. 252:2143-2153). Negative control cells (i.e., host cells from which the transfectants were prepared) are also included in the assay. The assay is conducted as follows:

Just prior to being assayed, transfected cells are removed from plates by scraping. Positive control cells used are PC12, BC3H1, and IMR32 (which had been starved for fresh media for seven days). Control cell lines are removed by rinsing in 37°C assay buffer (50mM Tris/HCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 120 mM NaCl, 3 mM EDTA, 2 mg/ml BSA and 0.1 % aprotinin at pH7.4). The cells are washed and resuspended to a concentration of 1 x 10^6/250 µl. To each plastic assay tube is added 250 µl of the cell solution, 15 nM ^{3}H-nicotine, with or without 1 mM unlabeled nicotine, and assay buffer to make a final volume of 500 µl. The assays for the transfected cell lines are incubated for 30 min at room temperature; the assays of the positive control cells are incubated for 2 min at 1°C. After the appropriate incubation time, 450 µl aliquots of assay volume are filtered through Whatman GF/C glass fiber filters which has been pretreated by incubation in 0.05% polyethyleneimine for 24 hours at

4°C. The filters are then washed twice, with 4 ml each wash, with ice cold assay buffer. After washing, the filters are dried, added to vials containing 5 ml scintillation fluid and radioactivity is measured.

C. ^{86}Rb ion-flux assay

The ability of nicotine or nicotine agonists and antagonists to mediate the influx of ^{86}Rb into transfected and control cells has been found to provide an indication of the presence of functional AChRs on the cell surface. The ^{86}Rb ion-flux assay is conducted as follows:

1. The night before the experiment, cells are plated at 2 x 10^6 per well (i.e., 2 ml per well) in a 6-well polylysine-coated plate.

2. The culture medium is decanted and the plate washed with 2 ml of assay buffer (50 mM HEPES, 260 mM sucrose, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, 5.5. mM glucose) at room temperature.

3. The assay buffer is decanted and 1 ml of assay buffer, containing 3 μCi/ml ^{86}Rb, with 5 mM ouabain and agonist or antagonist in a concentration to effect a maximum response, is added.

4. The plate is incubated on ice at 1°C for 4 min.

5. The buffer is decanted into a waste container and each well was washed with 3 ml of assay buffer, followed by two washes of 2 ml each.

6. The cells are lysed with 2 x 0.5 ml of 0.2% SDS per well and transferred to a scintillation vial containing 5 ml of scintillation fluid.

7. The radioactivity contained in each vial is measured and the data calculated.

Positive control cells provided the following data in this assay:

	PC12		IMR32	
	EC_{50}	Maximum response	EC_{50}	Maximum response
Agonist				
nicotine	52 μM	2.1X[a]	18 μM	7.7X[a]
CCh*	35 μM	3.3X[b]	230 μM	7.6X[c]
cytisine	57 μM	3.6X[d]	14 μM	10X[e]
Antagonist				
d-tubocurarine	0.81 μM		2.5 μM	
mecamylamine	0.42 μM		0.11 μM	
hexamethonium	nd[f]		22 μM	
atropine	12.5 μM		43 μM	

* CCh = carbamylcholine
[a] 200μM nicotine
[b] 300μM CCh
[c] 3mM CCh
[d] 1mM cytisine
[e] 100μM cytisine
[f] nd = not determined

D. Electrophysiological Analysis of Mammalian Cells Transfected with Human Neuronal Nicotinic AChR Subunit-encoding DNA

Electrophysiological measurements may be used to assess the activity of recombinant receptors or to assess the ability of a test compound to potentiate, antagonize or otherwise modulate the magnitude and duration of the flow of cations through the ligand-gated recombinant AChR. The function of the expressed neuronal AChR can be assessed by a variety of electrophysiological techniques, including two-electrode voltage clamp and patch clamp methods. The cation-conducting channel intrinsic to the AChR opens in response to acetylcholine (ACh) or other nicotinic cholinergic agonists, permitting the flow of transmembrane current carried predominantly by sodium and potassium ions under physiological conditions. This current can be monitored directly by voltage clamp techniques. In preferred embodiments, transfected mammalian cells or injected oocytes are analyzed electrophysiologically for the presence of AChR agonist-dependent currents.

While the invention has been described in detail with reference to certain preferred embodiments thereof, it will be understood that modifications and variations are within the spirit and scope of that which is described and claimed.

Summary of Sequences

Sequence ID No. 1 is a nucleotide sequence encoding an α_2 subunit of human neuronal nicotinic acetylcholine receptor.

Sequence ID No. 2 is the amino acid sequence deduced from the nucleotide sequence encoding an α_2 subunit of human neuronal nicotinic acetylcholine receptor set forth in Sequence ID No. 1.

Sequence ID No. 3 is a nucleotide sequence encoding an α_3 subunit of human neuronal nicotinic acetylcholine receptor.

Sequence ID No. 4 is the amino acid sequence deduced from the nucleotide sequence encoding an α_3 subunit of human neuronal nicotinic acetylcholine receptor set forth in Sequence ID No. 3.

Sequence ID No. 5 is a nucleotide sequence encoding an α_4 subunit of a human neuronal nicotinic acetylcholine receptor.

Sequence ID No. 6 is the amino acid sequence deduced from the nucleotide sequence encoding an α_4 subunit of a human neuronal nicotinic acetylcholine receptor set forth in Sequence ID No. 5.

Sequence ID No. 7 is a nucleotide sequence encoding an α_7 subunit of human neuronal nicotinic acetylcholine receptor.

Sequence ID No. 8 is the amino acid sequence deduced from the nucleotide sequence encoding an α_7 subunit of human neuronal nicotinic acetylcholine receptor set forth in Sequence ID No. 7.

Sequence ID No. 9 is a nucleotide sequence encoding a β_2 subunit of human neuronal nicotinic acetylcholine receptor.

Sequence ID No. 10 is the amino acid sequence deduced from the nucleotide sequence encoding a β_2 subunit of human neuronal nicotinic acetylcholine receptor set forth in Sequence ID No. 9.

Sequence ID No. 11 is a nucleotide sequence encoding a β_4 subunit of human neuronal nicotinic acetylcholine receptor.

Sequence ID No. 12 is the amino acid sequence deduced from the nucleotide sequence encoding a β_4 subunit of human neuronal nicotinic acetylcholine receptor set forth in Sequence ID No. 11.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Elliot, Kathryn J.
Ellis, Steven B.
Harpold, Michael M.

(ii) TITLE OF INVENTION: HUMAN NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR COMPOSITIONS AND METHODS EMPLOYING SAME

(iii) NUMBER OF SEQUENCES: 12

(iv) CORRESPONDENCE ADDRESS:
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(B) STREET: 444 South Flower Street, Suite 2000
(C) CITY: Los Angeles
(D) STATE: CA
(E) COUNTRY: USA
(F) ZIP: 90071

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE: 08-MAR-1994
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/028,031
(B) FILING DATE: 08-MAR-1993

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Reiter, Stephen E.
(B) REGISTRATION NUMBER: 31,192
(C) REFERENCE/DOCKET NUMBER: FP41 9714

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 619-546-4737
(B) TELEFAX: 619-546-9392

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2277 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 166..1755
(D) OTHER INFORMATION: /product= "ALPHA-2 SUBUNIT"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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GTTCTCTACA ACAATGCAGA TGGGGAGTTT GCAGTGACCC ACATGACCAA GGCCCACCTC 660

TTCTCCACGG GCACTGTGCA CTGGGTGCCC CCGGCCATCT ACAAGAGCTC CTGCAGCATC 720

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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 529 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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35 40 45

Leu Pro Gln Gly Gly Ser His Thr Glu Thr Glu Asp Arg Leu Phe Lys
50 55 60

His Leu Phe Arg Gly Tyr Asn Arg Trp Ala Arg Pro Val Pro Asn Thr
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Gln Glu Trp Ser Asp Tyr Lys Leu Arg Trp Asn Pro Ala Asp Phe Gly
        115                 120                 125

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    130                 135                 140

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                165                 170                 175

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                245                 250                 255

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    290                 295                 300

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                325                 330                 335

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            420                 425                 430

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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1757 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 39..1553
(D) OTHER INFORMATION: /product= "ALPHA-3 SUBUNIT"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 504 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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        35                  40                  45

Ile Arg Pro Val Ala Asn Val Ser Asp Pro Val Ile Ile His Phe Glu
    50                  55                  60

Val Ser Met Ser Gln Leu Val Lys Val Asp Glu Val Asn Gln Ile Met
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Glu Thr Asn Leu Trp Leu Lys Gln Ile Trp Asn Asp Tyr Lys Leu Lys
                85                  90                  95

Trp Asn Pro Ser Asp Tyr Gly Gly Ala Glu Phe Met Arg Val Pro Ala
            100                 105                 110

Gln Lys Ile Trp Lys Pro Asp Ile Val Leu Tyr Asn Asn Ala Val Gly
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(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2363 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 173..2056
(D) OTHER INFORMATION: /product= "ALPHA-4 SUBUNIT"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GCGCTCGCTG CGGCGCCGCC GCCGCNCCGC GCGCCACAGG AGAAGGCGAN CCGGGCCCGG 60

CGGCCGAAGC GGCCCGCGAG GCGCGGGAGG CATGAAGTTG GGCGCGCACG GGCCTCGAAG 120

CGGCGGGGAG CCGGGAGCCG CCCGCATCTA GAGCCCGCGA GGTGCGTGCG CCATGGAGCT 180

AGGGGGCCCC GGAGCGCCGC GGCTGCTGCC GCCGCTGCTG CTGCTTCTGG GGACCGGCCT 240

CCTGCGCGCC AGCAGCCATG TGGAGACCCG GGCCCACGCC GAGGAGCGGC TCCTGAAGAA 300

ACTCTTCTCC GGTTACAACA AGTGGTCCCG ACCCGTGGCC AACATCTCGG ACGTGGTCCT 360

CGTCCGCTTC GGCCTGTCCA TCGCTCAGCT CATTGACGTG GATGAGAAGA ACCAGATGAT 420

GACCACGAAC GTCTGGGTGA AGCAGGAGTG GCACGACTAC AAGCTGCGCT GGGACCCAGC 480

TGACTATGAG AATGTCACCT CCATCCGCAT CCCCTCCGAG CTCATCTGGC GGCCGGACAT 540

CGCCCTCTAC AACAATGCTG ACGGGGACTT CGCGGCCACC CACCTGACCA AGGCCCACCT 600

GTTCCATGAC GGGCGGGTGC AGCGGACTCC CCCGGCCATT TACAAGAGCT CCTGCAGCAT 660

CGACGTCACC TTCTTCCCCT TCGACCAGCA GAACTGCACC ATGAAATTCG GCTCCTGGAC 720

CTACGACAAG GCCAAGATCG ACCTGGTGAA CATGCACAGC CGCGTGGACC AGCTGGACTT 780

CTGGGAGAGT GGCGAGTGGC TCATCTCGGA CGCCGTGGGC ACCTACAACA CCAGGAAGTA 840

CGAGTGCTGC GCCGAGATCT ACCCGGACAT CACCTATGCC TACGCCATCC GGCGGCTGCC 900

GCTCTTCTAC ACCATCAACC TCATCATCCC CTGGCTGCTC ATCTCCTGCC TCACCGCGCT 960

GGTCTTCTAC CTGCCCTCCG AGTGTGGCGA GAAGATCACG CTGTGCATCT CCGTGCTGCT 1020

GTCGCTCACC GTCTTCCTGC TGCTCATCAC CGAGATCATC CCGTCCACCT CACTGGTCAT 1080

CCCACTCATC GGCGAGTACC TGCTGTTCAC CATGATCTTC GTCACCCTGT CCATCGCCAT 1140

CACGGTCTTC GTGCTCAACG TGCACCACCG CTCGCCACGC ACGCACACCA TGCCCACCTG 1200

GGTACGCAGG GTCTTCCTGG ACATCGTGCC ACGCCTGCTC CTCATGAAGC GGCCGTCCGT 1260

GGTCAAGGAC AATTGCCGGC GGCTCATCGA GTCCATGCAT AAGATGGCCA GTGCCCCGCG 1320

CTTCTGGCCC GAGCCAGAAG GGGAGCCCCC TGCCACGAGC GGCACCCAGA GCCTGCACCC 1380

TCCCTCACCG TCCTTCTGCG TCCCCCTGGA TGTGCCGGCT GAGCCTGGGC CTTCCTGCAA 1440

GTCACCCTCC GACCAGCTCC CTCCTCAGCA GCCCCTGGAA GCTGAGAAAG CCAGCCCCCA 1500

CCCCTCGCCT GGACCCTGCC GCCCGCCCCA CGGCACCCAG GCACCAGGGC TGGCCAAAGC 1560

CAGGTCCCTC AGCGTCCAGC ACATGTCCAG CCCTGGCGAA GCGGTGGAAG GCGGCGTCCG 1620

GTGCCGGTCT CGGAGCATCC AGTACTGTGT TCCCCGAGAC GATGCCGCCC CCGAGGCAGA 1680

TGGCCAGGCT GCCGGCGCCC TGGCCTCTCG CAACAGCCAC TCGGCTGAGC TCCCACCCCC 1740

AGACCAGCCC TCTCCGTGCA AATGCACATG CAAGAAGGAG CCCTCTTCGG TGTCCCCGAG 1800

CGCCACGGTC AAGACCCGCA GCACCAAAGC GCCGCCGCCG CACCTGCCCC TGTCGCCGGC 1860

CCTGAGCCGG GCGGTGGAGG GCGTCCAGTA CATTGCAGAC CACCTGAAGG CCGAAGACAC 1920

AGACTTCTCG GTGAAGGAGG ACTGGAAGTA CGTGGCCATG GTCATCGACC GCATCTTCCT 1980

CTGGATGTTC ATCATCGTCT GCCTGCTGGG GACGGTGGGC CTCTTCCTGC CGCCCTGGCT 2040

GGCTGGCATG ATCTAGGAAG GGACCGGGAG CCTGCGTGGC CTGGGGCTGC CGYGCACGGG 2100

GCCAGCATCC ATGCGGCCGG CCTGGGGCCG GGCTGGCTTC TCCCTGGACT CTGTGGGGCC 2160

ACACGTTTGC CAAATTTTCC TTCCTGTTCT GTGTCTGCTG TAAGACGGCC TTGGACGGCG 2220

ACACGGCCTC TGGGGAGACC GAGTGTGGAG CTGCTTCCAG TTGGACTCTS GCCTCAGNAG 2280

GCAGCGGCTT GGAGCAGAGG TGGCGGTCGC CGCCTYCTAC CTGCAGGACT CGGGCTAAGT 2340

CCAGCTCTCC CCCTGCGCAG CCC 2363

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 627 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Met Glu Leu Gly Gly Pro Gly Ala Pro Arg Leu Leu Pro Pro Leu Leu
1               5                  10                  15

Leu Leu Leu Gly Thr Gly Leu Leu Arg Ala Ser Ser His Val Glu Thr
            20                  25                  30

Arg Ala His Ala Glu Glu Arg Leu Leu Lys Lys Leu Phe Ser Gly Tyr
        35                  40                  45

Asn Lys Trp Ser Arg Pro Val Ala Asn Ile Ser Asp Val Val Leu Val
    50                  55                  60

Arg Phe Gly Leu Ser Ile Ala Gln Leu Ile Asp Val Asp Glu Lys Asn
65                  70                  75                  80

Gln Met Met Thr Thr Asn Val Trp Val Lys Gln Glu Trp His Asp Tyr
                85                  90                  95

Lys Leu Arg Trp Asp Pro Ala Asp Tyr Glu Asn Val Thr Ser Ile Arg
            100                 105                 110

Ile Pro Ser Glu Leu Ile Trp Arg Pro Asp Ile Ala Leu Tyr Asn Asn
        115                 120                 125

Ala Asp Gly Asp Phe Ala Ala Thr His Leu Thr Lys Ala His Leu Phe
    130                 135                 140

His Asp Gly Arg Val Gln Arg Thr Pro Pro Ala Ile Tyr Lys Ser Ser
145                 150                 155                 160

Cys Ser Ile Asp Val Thr Phe Phe Pro Phe Asp Gln Gln Asn Cys Thr
                165                 170                 175

Met Lys Phe Gly Ser Trp Thr Tyr Asp Lys Ala Lys Ile Asp Leu Val
            180                 185                 190

Asn Met His Ser Arg Val Asp Gln Leu Asp Phe Trp Glu Ser Gly Glu
        195                 200                 205

Trp Leu Ile Ser Asp Ala Val Gly Thr Tyr Asn Thr Arg Lys Tyr Glu
    210                 215                 220
```

```
Cys Cys Ala Glu Ile Tyr Pro Asp Ile Thr Tyr Ala Tyr Ala Ile Arg
225                 230                 235                 240

Arg Leu Pro Leu Phe Tyr Thr Ile Asn Leu Ile Ile Pro Trp Leu Leu
                245                 250                 255

Ile Ser Cys Leu Thr Ala Leu Val Phe Tyr Leu Pro Ser Glu Cys Gly
            260                 265                 270

Glu Lys Ile Thr Leu Cys Ile Ser Val Leu Leu Ser Leu Thr Val Phe
        275                 280                 285

Leu Leu Leu Ile Thr Glu Ile Ile Pro Ser Thr Ser Leu Val Ile Pro
    290                 295                 300

Leu Ile Gly Glu Tyr Leu Leu Phe Thr Met Ile Phe Val Thr Leu Ser
305                 310                 315                 320

Ile Ala Ile Thr Val Phe Val Leu Asn Val His His Arg Ser Pro Arg
                325                 330                 335

Thr His Thr Met Pro Thr Trp Val Arg Arg Val Phe Leu Asp Ile Val
            340                 345                 350

Pro Arg Leu Leu Leu Met Lys Arg Pro Ser Val Val Lys Asp Asn Cys
        355                 360                 365

Arg Arg Leu Ile Glu Ser Met His Lys Met Ala Ser Ala Pro Arg Phe
    370                 375                 380

Trp Pro Glu Pro Glu Gly Glu Pro Pro Ala Thr Ser Gly Thr Gln Ser
385                 390                 395                 400

Leu His Pro Pro Ser Pro Ser Phe Cys Val Pro Leu Asp Val Pro Ala
                405                 410                 415

Glu Pro Gly Pro Ser Cys Lys Ser Pro Ser Asp Gln Leu Pro Pro Gln
            420                 425                 430

Gln Pro Leu Glu Ala Glu Lys Ala Ser Pro His Pro Ser Pro Gly Pro
        435                 440                 445

Cys Arg Pro Pro His Gly Thr Gln Ala Pro Gly Leu Ala Lys Ala Arg
    450                 455                 460

Ser Leu Ser Val Gln His Met Ser Ser Pro Gly Glu Ala Val Glu Gly
465                 470                 475                 480

Gly Val Arg Cys Arg Ser Arg Ser Ile Gln Tyr Cys Val Pro Arg Asp
                485                 490                 495

Asp Ala Ala Pro Glu Ala Asp Gly Gln Ala Ala Gly Ala Leu Ala Ser
            500                 505                 510

Arg Asn Ser His Ser Ala Glu Leu Pro Pro Pro Asp Gln Pro Ser Pro
        515                 520                 525
```

Cys Lys Cys Thr Cys Lys Lys Glu Pro Ser Ser Val Ser Pro Ser Ala
530 535 540

Thr Val Lys Thr Arg Ser Thr Lys Ala Pro Pro Pro His Leu Pro Leu
545 550 555 560

Ser Pro Ala Leu Ser Arg Ala Val Glu Gly Val Gln Tyr Ile Ala Asp
565 570 575

His Leu Lys Ala Glu Asp Thr Asp Phe Ser Val Lys Glu Asp Trp Lys
580 585 590

Tyr Val Ala Met Val Ile Asp Arg Ile Phe Leu Trp Met Phe Ile Ile
595 600 605

Val Cys Leu Leu Gly Thr Val Gly Leu Phe Leu Pro Pro Trp Leu Ala
610 615 620

Gly Met Ile
625

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1876 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 73..1581
(D) OTHER INFORMATION: /product= "ALPHA-7 SUBUNIT"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GGCCGCAGGC GCAGGCCCGG GCGACAGCCG AGACGTGGAG CGCGCCGGCT CGCTGCAGCT 60

CCGGGACTCA ACATGCGCTG CTCGCCGGGA GGCGTCTGGC TGGCGCTGGC CGCGTCGCTC 120

CTGCACGTGT CCCTGCAAGG CGAGTTCCAG AGGAAGCTTT ACAAGGAGCT GGTCAAGAAC 180

TACAATCCCT TGGAGAGGCC CGTGGCCAAT GACTCGCAAC CACTCACCGT CTACTTCTCC 240

CTGAGCCTCC TGCAGATCAT GGACGTGGAT GAGAAGAACC AAGTTTTAAC CACCAACATT 300

TGGCTGCAAA TGTCTTGGAC AGATCACTAT TTACAGTGGA ATGTGTCAGA ATATCCAGGG 360

GTGAAGACTG TTCGTTTCCC AGATGGCCAG ATTTGGAAAC CAGACATTCT TCTCTATAAC 420

AGTGCTGATG AGCGCTTTGA CGCCACATTC CACACTAACG TGTTGGTGAA TTCTTCTGGG 480

CATTGCCAGT ACCTGCCTCC AGGCATATTC AAGAGTTCCT GCTACATCGA TGTACGCTGG 540

TTTCCCTTTG ATGTGCAGCA CTGCAAACTG AAGTTTGGGT CCTGGTCTTA CGGAGGCTGG 600

TCCTTGGATC TGCAGATGCA GGAGGCAGAT ATCAGTGGCT ATATCCCCAA TGGAGAATGG 660

GACCTAGTGG GAATCCCCGG CAAGAGGAGT GAAAGGTTCT ATGAGTGCTG CAAAGAGCCC 720

TACCCCGATG TCACCTTCAC AGTGACCATG CGCCGCAGGA CGCTCTACTA TGGCCTCAAC 780

CTGCTGATCC CCTGTGTGCT CATCTCCGCC CTCGCCCTGC TGGTGTTCCT GCTTCCTGCA 840

GATTCCGGGG AGAAGATTTC CCTGGGGATA ACAGTCTTAC TCTCTCTTAC CGTCTTCATG 900

CTGCTCGTGG CTGAGATCAT GCCCGCAACA TCCGATTCGG TACCATTGAT AGCCCAGTAC 960

TTCGCCAGCA CCATGATCAT CGTGGGCCTC TCGGTGGTGG TGACGGTGAT CGTGCTGCAG 1020

TACCACCACC ACGACCCCGA CGGGGGCAAG ATGCCCAAGT GGACCAGAGT CATCCTTCTG 1080

AACTGGTGCG CGTGGTTCCT SCGAATGAAG AGGCCCGGGG AGGACAAGGT GCGCCCGGCC 1140

TGCCAGCACA AGCAGCGGCG CTGCAGCCTG GCCAGTGTGG AGATGAGCGC CGTGGCGCCG 1200

CCGCCCGCCA GCAACGGGAA CCTGCTGTAC ATCGGCTTCC GCGGCCTGGA CGGCGTGCAC 1260

TGTGTCCCGA CCCCCGACTC TGGGGTAGTG TGTGGCCGCA TGGCCTGCTC CCCCACGCAC 1320

GATGAGCACC TCCTGCACGG CGGGCAACCC CCCGAGGGGG ACCCGGACTT GGCCAAGATC 1380

CTGGAGGAGG TCCGCTACAT TGCCAATCGC TTCCGCTGCC AGGACGAAAG CGAGGCGGTC 1440

TGCAGCGAGT GGAAGTTCGC CGCCTGTGTG GTGGACCGCC TGTGCCTCAT GGCCTTCTCG 1500

GTCTTCACCA TCATCTGCAC CATCGGCATC CTGATGTCGG CTCCCAACTT CGTGGAGGCC 1560

GTGTCCAAAG ACTTTGCGTA ACCACGCCTG GTTCTGTACA TGTGGAAAAC TCACAGATGG 1620

GCAAGGCCTT TGGCTTGGCG AGATTTGGGG GTGCTAATCC AGGACAGCAT TACACGCCAC 1680

AACTCCAGTG TTCCCTTCTG GCTGTCAGTC GTGTTGCTTA CGGTTTCTTT GTTACTTTAG 1740

GTAGTAGAAT CTCAGCACTT TGTTTCATAT TCTCAGATGG GCTGATAGAT ATCCTTGGCA 1800

CATCCGTACC ATCGGTCAGC AGGGCCACTG AGTAGTCATT TTGCCCATTA GCCCACTGCC 1860

TGGAAAGCCC TTCGGA 1876

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 502 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Met Arg Cys Ser Pro Gly Gly Val Trp Leu Ala Leu Ala Ala Ser Leu
1               5                  10                  15

Leu His Val Ser Leu Gln Gly Glu Phe Gln Arg Lys Leu Tyr Lys Glu
            20                  25                  30

Leu Val Lys Asn Tyr Asn Pro Leu Glu Arg Pro Val Ala Asn Asp Ser
        35                  40                  45

Gln Pro Leu Thr Val Tyr Phe Ser Leu Ser Leu Leu Gln Ile Met Asp
    50                  55                  60

Val Asp Glu Lys Asn Gln Val Leu Thr Thr Asn Ile Trp Leu Gln Met
65                  70                  75                  80

Ser Trp Thr Asp His Tyr Leu Gln Trp Asn Val Ser Glu Tyr Pro Gly
                85                  90                  95

Val Lys Thr Val Arg Phe Pro Asp Gly Gln Ile Trp Lys Pro Asp Ile
            100                 105                 110

Leu Leu Tyr Asn Ser Ala Asp Glu Arg Phe Asp Ala Thr Phe His Thr
        115                 120                 125

Asn Val Leu Val Asn Ser Ser Gly His Cys Gln Tyr Leu Pro Pro Gly
    130                 135                 140

Ile Phe Lys Ser Ser Cys Tyr Ile Asp Val Arg Trp Phe Pro Phe Asp
145                 150                 155                 160

Val Gln His Cys Lys Leu Lys Phe Gly Ser Trp Ser Tyr Gly Gly Trp
                165                 170                 175

Ser Leu Asp Leu Gln Met Gln Glu Ala Asp Ile Ser Gly Tyr Ile Pro
            180                 185                 190

Asn Gly Glu Trp Asp Leu Val Gly Ile Pro Gly Lys Arg Ser Glu Arg
        195                 200                 205

Phe Tyr Glu Cys Cys Lys Glu Pro Tyr Pro Asp Val Thr Phe Thr Val
    210                 215                 220
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Thr Met Arg Arg Arg Thr Leu Tyr Tyr Gly Leu Asn Leu Leu Ile Pro
225                 230                 235                 240

Cys Val Leu Ile Ser Ala Leu Ala Leu Leu Val Phe Leu Leu Pro Ala
                245                 250                 255

Asp Ser Gly Glu Lys Ile Ser Leu Gly Ile Thr Val Leu Leu Ser Leu
            260                 265                 270

Thr Val Phe Met Leu Leu Val Ala Glu Ile Met Pro Ala Thr Ser Asp
        275                 280                 285

Ser Val Pro Leu Ile Ala Gln Tyr Phe Ala Ser Thr Met Ile Ile Val
    290                 295                 300

Gly Leu Ser Val Val Val Thr Val Ile Val Leu Gln Tyr His His His
305                 310                 315                 320

Asp Pro Asp Gly Gly Lys Met Pro Lys Trp Thr Arg Val Ile Leu Leu
                325                 330                 335

Asn Trp Cys Ala Trp Phe Leu Arg Met Lys Arg Pro Gly Glu Asp Lys
            340                 345                 350

Val Arg Pro Ala Cys Gln His Lys Gln Arg Arg Cys Ser Leu Ala Ser
        355                 360                 365

Val Glu Met Ser Ala Val Ala Pro Pro Pro Ala Ser Asn Gly Asn Leu
    370                 375                 380

Leu Tyr Ile Gly Phe Arg Gly Leu Asp Gly Val His Cys Val Pro Thr
385                 390                 395                 400

Pro Asp Ser Gly Val Val Cys Gly Arg Met Ala Cys Ser Pro Thr His
                405                 410                 415

Asp Glu His Leu Leu His Gly Gly Gln Pro Pro Glu Gly Asp Pro Asp
            420                 425                 430

Leu Ala Lys Ile Leu Glu Glu Val Arg Tyr Ile Ala Asn Arg Phe Arg
        435                 440                 445

Cys Gln Asp Glu Ser Glu Ala Val Cys Ser Glu Trp Lys Phe Ala Ala
    450                 455                 460

Cys Val Val Asp Arg Leu Cys Leu Met Ala Phe Ser Val Phe Thr Ile
465                 470                 475                 480

Ile Cys Thr Ile Gly Ile Leu Met Ser Ala Pro Asn Phe Val Glu Ala
                485                 490                 495

Val Ser Lys Asp Phe Ala
            500
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2448 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 265..1773
(D) OTHER INFORMATION: /product= "BETA-2 SUBUNIT"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CTCCTCCCCC TCACCGTCCC AATTGTATTC CCTGGAAGAG CAGCCGGAAA AGCCTCCGCC 60

TGCTCATACC AGGATAGGCA AGAAGCTGGT TTCTCCTCGC AGCCGGCTCC CTGAGGCCCA 120

GGAACCACCG CGGCGGCCGG CACCACCTGG ACCCAGCTCC AGGCGGGCGC GGCTTCAGCA 180

CCACGGACAG CGCCCCACCC GCGGCCCTCC CCCCGGCGGC GCGCTCCAGC CGGTGTAGGC 240

GAGGCAGCGA GCTATGCCCG CGGCATGGCC CGGCGCTGCG GCCCCGTGGC GCTGCTCCTT 300

GGCTTCGGCC TCCTCCGGCT GTGCTCAGGG GTGTGGGGTA CGGATACAGA GGAGCGGCTG 360

GTGGAGCATC TCCTGGATCC TTCCCGCTAC AACAAGCTTA TCCGCCCAGC CACCAATGGC 420

TCTGAGCTGG TGACAGTACA GCTTATGGTG TCACTGGCCC AGCTCATCAG TGTGCATGAG 480

CGGGAGCAGA TCATGACCAC CAATGTCTGG CTGACCCAGG AGTGGGAAGA TTATCGCCTC 540

ACCTGGAAGC CTGAAGAGTT TGACAACATG AAGAAAGTTC GGCTCCCTTC CAAACACATC 600

TGGCTCCCAG ATGTGGTCCT GTACAACAAT GCTGACGGCA TGTACGAGGT GTCCTTCTAT 660

TCCAATGCCG TGGTCTCCTA TGATGGCAGC ATCTTCTGGC TGCCGCCTGC CATCTACAAG 720

AGCGCATGCA AGATTGAAGT AAAGCACTTC CCATTTGACC AGCAGAACTG CACCATGAAG 780

TTCCGTTCGT GGACCTACGA CCGCACAGAG ATCGACTTGG TGCTGAAGAG TGAGGTGGCC 840

AGCCTGGACG ACTTCACACC TAGTGGTGAG TGGGACATCG TGGCGCTGCC GGGCCGGCGC 900

AACGAGAACC CCGACGACTC TACGTACGTG GACATCACGT ATGACTTCAT CATTCGCCGC 960

AAGCCGCTCT TCTACACCAT CAACCTCATC ATCCCCTGTG TGCTCATCAC CTCGCTAGCC 1020

ATCCTTGTCT TCTACCTGCC ATCCGACTGT GGCGAGAAGA TGACGTTGTG CATCTCAGTG 1080

CTGCTGGCGC TCACGGTCTT CCTGCTGCTC ATCTCCAAGA TCGTGCCTCC CACCTCCCTC 1140

GACGTGCCGC TCGTCGGCAA GTACCTCATG TTCACCATGG TGCTTGTCAC CTTCTCCATC 1200

GTCACCAGCG TGTGCGTGCT CAACGTGCAC CACCGCTCGC CCACCACGCA CACCATGGCG 1260

CCCTGGGTGA AGGTCGTCTT CCTGGAGAAG CTGCCCGCGC TGCTCTTCAT GCAGCAGCCA 1320

CGCCATCATT GCGCCCGTCA GCGCCTGCGC CTGCGGCGAC GCCAGCGTGA GCGCGAGGGC 1380

GCTGGAGCCC TCTTCTTCCG CGAAGCCCCA GGGGCCGACT CCTGCACGTG CTTCGTCAAC 1440

CGCGCGTCGG TGCAGGGGTT GGCCGGGGCC TTCGGGGCTG AGCCTGCACC AGTGGCGGGC 1500

CCCGGGCGCT CAGGGGAGCC GTGTGGCTGT GGCCTCCGGG AGGCGGTGGA CGGCGTGCGC 1560

TTCATCGCAG ACCACATGCG GAGCGAGGAC GATGACCAGA GCGTGAGTGA GGACTGGAAG 1620

TACGTCGCCA TGGTGATCGA CCGCCTCTTC CTCTGGATCT TTGTCTTTGT CTGTGTCTTT 1680

GGCACCATCG GCATGTTCCT GCAGCCTCTC TTCCAGAACT ACACCACCAC CACCTTCCTC 1740

CACTCAGACC ACTCAGCCCC CAGCTCCAAG TGAGGCCCTT CCTCATCTCC ATGCTCTTTC 1800

ACCCTGCCAC CCTCTGCTGC ACAGTAGTGT TGGGTGGAGG ATGGACGAGT GAGCTACCAG 1860

GAAGAGGGGC GCTGCCCCCA CAGATCCATC CTTTTGCTTC ATCTGGAGTC CCTCCTCCCC 1920

CACGCCTCCA TCCACACACA GCAGCTCCAA CCTGGAGGCT GGACCAACTG CTTTGTTTTG 1980

GCTGCTCTCC ATCTCTTGTA CCAGCCCAGG CAATAGTGTT GAGGAGGGGA GCAAGGCTGC 2040

TAAGTGGAAG ACAGAGATGG CAGAGCCATC CACCCTGAGG AGTGACGGGC AAGGGGCCAG 2100

GAAGGGGACA GGATTGTCTG CTGCCTCCAA GTCATGGGAG AAGAGGGGTA TAGGACAAGG 2160

GGTGGAAGGG CAGGAGCTCA CACCGCACCG GGCTGGCCTG ACACAATGGT AGCTCTGAAG 2220

GGAGGGGAAG AGAGAGGCCT GGGTGTGACC TGACACCTGC CGCTGCTTGA GTGGACAGCA 2280

GCTGGACTGG GTGGGCCCCA CAGTGGTCAG CGATTCCTGC CAAGTAGGGT TTAGCCGGGC 2340

CCCATGGTCA CAGACCCCTG GGGGAGGCTT CCAGCTCAGT CCCACAGCCC CTTGCTTCTA 2400

AGGGATCCAG AGACCTGCTC CAGATCCTCT TTCCCCACTG AAGAATTC 2448

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 502 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Ala Arg Arg Cys Gly Pro Val Ala Leu Leu Leu Gly Phe Gly Leu
1 5 10 15

Leu Arg Leu Cys Ser Gly Val Trp Gly Thr Asp Thr Glu Glu Arg Leu
20 25 30

Val Glu His Leu Leu Asp Pro Ser Arg Tyr Asn Lys Leu Ile Arg Pro
35 40 45

Ala Thr Asn Gly Ser Glu Leu Val Thr Val Gln Leu Met Val Ser Leu
50 55 60

Ala Gln Leu Ile Ser Val His Glu Arg Glu Gln Ile Met Thr Thr Asn
65 70 75 80

Val Trp Leu Thr Gln Glu Trp Glu Asp Tyr Arg Leu Thr Trp Lys Pro
85 90 95

Glu Glu Phe Asp Asn Met Lys Lys Val Arg Leu Pro Ser Lys His Ile
100 105 110

Trp Leu Pro Asp Val Val Leu Tyr Asn Asn Ala Asp Gly Met Tyr Glu
115 120 125

Val Ser Phe Tyr Ser Asn Ala Val Val Ser Tyr Asp Gly Ser Ile Phe
130 135 140

Trp Leu Pro Pro Ala Ile Tyr Lys Ser Ala Cys Lys Ile Glu Val Lys
145 150 155 160

His Phe Pro Phe Asp Gln Gln Asn Cys Thr Met Lys Phe Arg Ser Trp
165 170 175

Thr Tyr Asp Arg Thr Glu Ile Asp Leu Val Leu Lys Ser Glu Val Ala
180 185 190

Ser Leu Asp Asp Phe Thr Pro Ser Gly Glu Trp Asp Ile Val Ala Leu
195 200 205

Pro Gly Arg Arg Asn Glu Asn Pro Asp Asp Ser Thr Tyr Val Asp Ile
210 215 220

```
Thr Tyr Asp Phe Ile Ile Arg Arg Lys Pro Leu Phe Tyr Thr Ile Asn
225                 230                 235                 240

Leu Ile Ile Pro Cys Val Leu Ile Thr Ser Leu Ala Ile Leu Val Phe
                245                 250                 255

Tyr Leu Pro Ser Asp Cys Gly Glu Lys Met Thr Leu Cys Ile Ser Val
            260                 265                 270

Leu Leu Ala Leu Thr Val Phe Leu Leu Leu Ile Ser Lys Ile Val Pro
        275                 280                 285

Pro Thr Ser Leu Asp Val Pro Leu Val Gly Lys Tyr Leu Met Phe Thr
    290                 295                 300

Met Val Leu Val Thr Phe Ser Ile Val Thr Ser Val Cys Val Leu Asn
305                 310                 315                 320

Val His His Arg Ser Pro Thr Thr His Thr Met Ala Pro Trp Val Lys
                325                 330                 335

Val Val Phe Leu Glu Lys Leu Pro Ala Leu Leu Phe Met Gln Gln Pro
            340                 345                 350

Arg His His Cys Ala Arg Gln Arg Leu Arg Leu Arg Arg Arg Gln Arg
        355                 360                 365

Glu Arg Glu Gly Ala Gly Ala Leu Phe Phe Arg Glu Ala Pro Gly Ala
    370                 375                 380

Asp Ser Cys Thr Cys Phe Val Asn Arg Ala Ser Val Gln Gly Leu Ala
385                 390                 395                 400

Gly Ala Phe Gly Ala Glu Pro Ala Pro Val Ala Gly Pro Gly Arg Ser
                405                 410                 415

Gly Glu Pro Cys Gly Cys Gly Leu Arg Glu Ala Val Asp Gly Val Arg
            420                 425                 430

Phe Ile Ala Asp His Met Arg Ser Glu Asp Asp Asp Gln Ser Val Ser
        435                 440                 445

Glu Asp Trp Lys Tyr Val Ala Met Val Ile Asp Arg Leu Phe Leu Trp
    450                 455                 460

Ile Phe Val Phe Val Cys Val Phe Gly Thr Ile Gly Met Phe Leu Gln
465                 470                 475                 480

Pro Leu Phe Gln Asn Tyr Thr Thr Thr Thr Phe Leu His Ser Asp His
                485                 490                 495

Ser Ala Pro Ser Ser Lys
            500
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1915 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 87..1583
(D) OTHER INFORMATION: /product= "BETA-4 SUBUNIT"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CCGGCGCTCA CTCGACCGCG CGGCTCACGG GTGCCCTGTG ACCCCACAGC GGAGCTCGCG 60

GCGGCTGCCA CCCGGCCCCG CCGGCCATGA GGCGCGCGCC TTCCCTGGTC CTTTTCTTCC 120

TGGTCGCCCT TTGCGGGCGC GGGAACTGCC GCGTGGCCAA TGCGGAGGAA AAGCTGATGG 180

ACGACCTTCT GAACAAAACC CGTTACAATA ACCTGATCCG CCCAGCCACC AGCTCCTCAC 240

AGCTCATCTC CATCAAGCTG CAGCTCTCCC TGGCCCAGCT TATCAGCGTG AATGAGCGAG 300

AGCAGATCAT GACCACCAAT GTCTGGCTGA AACAGGAATG GACTGATTAC CGCCTGACCT 360

GGAACAGCTC CCGCTACGAG GGTGTGAACA TCCTGAGGAT CCCTGCAAAG CGCATCTGGT 420

TGCCTGACAT CGTGCTTTAC AACAACGCCG ACGGGACCTA TGAGGTGTCT GTCTACACCA 480

ACTTGATAGT CCGGTCCAAC GGCAGCGTCC TGTGGCTGCC CCCTGCCATC TACAAGAGCG 540

CCTGCAAGAT TGAGGTGAAG TACTTTCCCT TCGACCAGCA GAACTGCACC CTCAAGTTCC 600

GCTCCTGGAC CTATGACCAC ACGGAGATAG ACATGGTCCT CATGACGCCC ACAGCCAGCA 660

TGGATGACTT TACTCCCAGT GGTGAGTGGG ACATAGTGGC CCTCCCAGGG AGAAGGACAG 720

TGAACCCACA AGACCCCAGC TACGTGGACG TGACTTACGA CTTCATCATC AAGCGCAAGC 780

CTCTGTTCTA CACCATCAAC CTCATCATCC CCTGCGTGCT CACCACCTTG CTGGCCATCC 840

TCGTCTTCTA CCTGCCATCC GACTGCGGCG AGAAGATGAC ACTGTGCATC TCAGTGCTGC 900

TGGCACTGAC ATTCTTCCTG CTGCTCATCT CCAAGATCGT GCCACCCACC TCCCTCGATG 960

TGCCTCTCAT CGGCAAGTAC CTCATGTTCA CCATGGTGCT GGTCACCTTC TCCATCGTCA 1020

CCAGCGTCTG TGTGCTCAAT GTGCACCACC GCTCGCCCAG CACCCACACC ATGGCACCCT 1080

GGGTCAAGCG CTGCTTCCTG CACAAGCTGC CTACCTTCCT CTTCATGAAG CGCCCTGGCC 1140

CCGACAGCAG CCCGGCCAGA GCCTTCCCGC CCAGCAAGTC ATGCGTGACC AAGCCCGAGG 1200

CCACCGCCAC CTCCACCAGC CCCTCCAACT TCTATGGGAA CTCCATGTAC TTTGTGAACC 1260

CCGCCTCTGC AGCTTCCAAG TCTCCAGCCG GCTCTACCCC GGTGGCTATC CCCAGGGATT 1320

TCTGGCTGCG GTCCTCTGGG AGGTTCCGAC AGGATGTGCA GGAGGCATTA GAAGGTGTCA 1380

GCTTCATCGC CCAGCACATG AAGAATGACG ATGAAGACCA GAGTGTCGTT GAGGACTGGA 1440

AGTACGTGGC TATGGTGGTG GACCGGCTGT TCCTGTGGGT GTTCATGTTT GTGTGCGTCC 1500

TGGGCACTGT GGGGCTCTTC CTGCCGCCCC TCTTCCAGAC CCATGCAGCT TCTGAGGGGC 1560

CCTACGCTGC CCAGCGTGAC TGAGGGCCCC CTGGGTTGTG GGGTGAGAGG ATGTGAGTGG 1620

CCGGGTGGGC ACTTTGCTGC TTCTTTCTGG GTTGTGGCCG ATGAGGCCCT AAGTAAATAT 1680

GTGAGCATTG GCCATCAACC CCATCAAACC AGCCACAGCC GTGGAACAGG CAAGGATGGG 1740

GGCCTGGCCT GTCCTCTCTG AATGCCTTGG AGGGATCCCA GGAAGCCCCA GTAGGAGGGA 1800

GCTTCAGACA GTTCAATTCT GGCCTGTCTT CCTTCCCTGC ACCGGGCAAT GGGGATAAAG 1860

ATGACTTCGT AGCAGCACCT ACTATGCTTC AGGCATGGTG CCGGCCTGCC TCTCC 1915

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 498 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Met Arg Arg Ala Pro Ser Leu Val Leu Phe Phe Leu Val Ala Leu Cys
1 5 10 15

Gly Arg Gly Asn Cys Arg Val Ala Asn Ala Glu Glu Lys Leu Met Asp
20 25 30

Asp Leu Leu Asn Lys Thr Arg Tyr Asn Asn Leu Ile Arg Pro Ala Thr
35 40 45

Ser Ser Ser Gln Leu Ile Ser Ile Lys Leu Gln Leu Ser Leu Ala Gln
50 55 60

Leu Ile Ser Val Asn Glu Arg Glu Gln Ile Met Thr Thr Asn Val Trp
65 70 75 80

Leu Lys Gln Glu Trp Thr Asp Tyr Arg Leu Thr Trp Asn Ser Ser Arg
85 90 95

Tyr Glu Gly Val Asn Ile Leu Arg Ile Pro Ala Lys Arg Ile Trp Leu
100 105 110

Pro Asp Ile Val Leu Tyr Asn Asn Ala Asp Gly Thr Tyr Glu Val Ser
115 120 125

Val Tyr Thr Asn Leu Ile Val Arg Ser Asn Gly Ser Val Leu Trp Leu
130 135 140

Pro Pro Ala Ile Tyr Lys Ser Ala Cys Lys Ile Glu Val Lys Tyr Phe
145 150 155 160

Pro Phe Asp Gln Gln Asn Cys Thr Leu Lys Phe Arg Ser Trp Thr Tyr
165 170 175

Asp His Thr Glu Ile Asp Met Val Leu Met Thr Pro Thr Ala Ser Met
180 185 190

Asp Asp Phe Thr Pro Ser Gly Glu Trp Asp Ile Val Ala Leu Pro Gly
195 200 205

Arg Arg Thr Val Asn Pro Gln Asp Pro Ser Tyr Val Asp Val Thr Tyr
210 215 220

Asp Phe Ile Ile Lys Arg Lys Pro Leu Phe Tyr Thr Ile Asn Leu Ile
225 230 235 240

Ile Pro Cys Val Leu Thr Thr Leu Leu Ala Ile Leu Val Phe Tyr Leu
245 250 255

Pro Ser Asp Cys Gly Glu Lys Met Thr Leu Cys Ile Ser Val Leu Leu
260 265 270

Ala Leu Thr Phe Phe Leu Leu Leu Ile Ser Lys Ile Val Pro Pro Thr
275 280 285

Ser Leu Asp Val Pro Leu Ile Gly Lys Tyr Leu Met Phe Thr Met Val
290 295 300

Leu Val Thr Phe Ser Ile Val Thr Ser Val Cys Val Leu Asn Val His
305 310 315 320

His Arg Ser Pro Ser Thr His Thr Met Ala Pro Trp Val Lys Arg Cys
325 330 335

Phe Leu His Lys Leu Pro Thr Phe Leu Phe Met Lys Arg Pro Gly Pro
340 345 350

Asp Ser Ser Pro Ala Arg Ala Phe Pro Pro Ser Lys Ser Cys Val Thr
355 360 365

Lys Pro Glu Ala Thr Ala Thr Ser Thr Ser Pro Ser Asn Phe Tyr Gly
370 375 380

Asn Ser Met Tyr Phe Val Asn Pro Ala Ser Ala Ala Ser Lys Ser Pro
385 390 395 400

Ala Gly Ser Thr Pro Val Ala Ile Pro Arg Asp Phe Trp Leu Arg Ser
405 410 415

Ser Gly Arg Phe Arg Gln Asp Val Gln Glu Ala Leu Glu Gly Val Ser
420 425 430

Phe Ile Ala Gln His Met Lys Asn Asp Asp Glu Asp Gln Ser Val Val
435 440 445

Glu Asp Trp Lys Tyr Val Ala Met Val Val Asp Arg Leu Phe Leu Trp
450 455 460

Val Phe Met Phe Val Cys Val Leu Gly Thr Val Gly Leu Phe Leu Pro
465 470 475 480

Pro Leu Phe Gln Thr His Ala Ala Ser Glu Gly Pro Tyr Ala Ala Gln
485 490 495

Arg Asp

THAT WHICH IS CLAIMED:

1. Isolated DNA comprising a sequence of nucleotides encoding an α_4 or α_7 subunit of a human neuronal nicotinic acetylcholine receptor.

2. DNA according to claim 1 wherein the subunit is an α_4 subunit.

3. DNA according to claim 2 wherein said DNA encodes:

the amino acid sequence set forth in SEQ ID No:6, or

the amino acid sequence encoded by clone HnAChRα4.2 (ATCC Accession No. 69239), or

the 5' nucleotides of said DNA encode the amino acid sequence encoded by clone HnAChRα4.1 (ATCC Accession No. 69152).

4. DNA according to claim 2 wherein said DNA hybridizes:

to substantially the entire coding sequence (nucleotides 173-2056) set forth in SEQ ID No:5 under high stringency conditions, or

under high stringency conditions to substantially the entire sequence of the α_4-encoding insert of clone HnAChRα4.2 (ATCC Accession No. 69239), or

the 5' nucleotides of said DNA hybridize under high stringency conditions to the sequence of the α_4-encoding insert of clone HnAChRα4.1 (ATCC Accession No. 69152).

5. DNA according to claim 2 wherein said DNA has substantially the same nucleotide sequence as:

nucleotides 173-2056 set forth in SEQ ID No:5, or

the α_4-encoding insert of clone HnAChRα4.2 (ATCC Accession No. 69239), or

the 5' nucleotides of said DNA have substantially the same sequence as the α_4-encoding insert of clone HnAChRα4.1 (ATCC Accession No. 69152).

6. DNA according to claim 1 wherein the subunit is an α_7 subunit.

7. DNA according to claim 6 wherein the nucleotides of said DNA encodes the amino acid sequence set forth in SEQ ID No:8.

8. DNA according to claim 6 wherein the nucleotides of said DNA hybridize under high stringency conditions to substantially the entire coding sequence (nucleotides 73-1581) set forth in SEQ ID No:7.

9. DNA according to claim 6 wherein the nucleotides of said DNA have substantially the same nucleotide sequence as nucleotides 73-1581 set forth in SEQ ID No:7.

10. Isolated DNA comprising nucleotides encoding a β_4 subunit of a human neuronal nicotinic acetylcholine receptor.

11. DNA according to claim 10 wherein the nucleotides of said DNA encode the amino acid sequence set forth in SEQ ID No:12.

12. DNA according to claim 10 wherein the nucleotides of said DNA hybridize under high stringency conditions to substantially the entire coding sequence (nucleotides 87-1583) set forth in SEQ ID No:11.

13. DNA according to claim 10 wherein the nucleotides of said DNA have substantially the same nucleotide sequence as nucleotides 87-1583 set forth in SEQ ID No:11.

14. Cells containing at least one DNA according to claim 1, wherein said cells are bacterial cells, eukaryotic cells or amphibian oöcytes.

15. Cells according to claim 14, further containing at least one DNA encoding a β subunit of human neuronal nicotinic acetylcholine receptor.

16. Cells according to claim 15 further characterized as being capable of expressing voltage dependent calcium channels.

17. Cells according to claim 15 wherein said β subunit is selected from β_2 or β_4.

18. Cells according to claim 15 wherein said β subunit is β_4.

19. Cells according to claim 14 wherein said cells express functional neuronal nicotinic acetylcholine receptors that contain one or more subunits encoded by said DNA.

20. Cells containing at least one DNA according to claim 10, wherein said cells are bacterial cells, eukaryotic cells or amphibian oöcytes.

21. Cells according to claim 20, further containing at least one DNA encoding an α subunit of human neuronal nicotinic acetylcholine receptor.

22. Cells according to claim 21 further characterized as being capable of expressing voltage dependent calcium channels.

23. Cells according to claim 21 wherein said α subunit is selected from α_1, α_2, α_3, α_4, α_5 or α_7.

24. Cells according to claim 21 wherein said α subunit is selected from α_4 or α_7.

25. Cells according to claim 21, containing DNA encoding human α_3 and human β_4 subunits.

26. Cells according to claim 20 wherein said cells express functional neuronal nicotinic acetylcholine receptors that contain one or more subunits encoded by said DNA.

27. A method of screening compounds to identify compounds which modulate the activity of human neuronal nicotinic acetylcholine receptors, said method comprising determining the effect of a compound on the neuronal nicotinic acetylcholine receptor activity in test cells according to claim 15, compared to the effect on control cells or to the neuronal nicotinic acetylcholine receptor activity of the cells in the absence of the compound,

wherein control cells are substantially identical to the test cells, but control cells do not express nicotinic acetylcholine receptors.

28. A method of screening compounds to identify compounds which modulate the activity of human neuronal nicotinic acetylcholine receptors, said method comprising determining the effect of a compound on the neuronal nicotinic acetylcholine receptor activity in test cells according to claim 21, compared to the effect on control cells or to the neuronal nicotinic acetylcholine receptor activity of the cells in the absence of the compound,

wherein control cells are substantially identical to the test cells, but control cells do not express nicotinic acetylcholine receptors.

29. A recombinant human neuronal nicotinic acetylcholine receptor subunit selected from an α_4 or α_7 subunit.

30. A recombinant human neuronal nicotinic acetylcholine receptor comprising one or more of the subunits of claim 29.

31. Human neuronal nicotinic acetylcholine receptor according to claim 30, further comprising at least one human neuronal nicotinic acetylcholine receptor beta subunit.

32. Recombinant human neuronal nicotinic acetylcholine receptor β_4 subunit.

33. A recombinant human neuronal nicotinic acetylcholine receptor comprising the subunit of claim 32.

34. Human neuronal nicotinic acetylcholine receptor according to claim 33, further comprising at least one human neuronal nicotinic acetylcholine receptor alpha subunit.

35. A method for identifying functional neuronal nicotinic acetylcholine receptor subunits and combinations thereof, said method comprising:

(a) introducing at least one DNA according to claim 1, or RNA complementary thereto, and optionally DNA encoding at least one beta subunit of a human neuronal nicotinic acetylcholine receptor, or RNA complementary thereto, into eukaryotic cells; and

(b) assaying for neuronal nicotinic acetylcholine receptor activity in cells of step (a), wherein the activity is mediated by a receptor containing one or more of the subunits encoded by said introduced DNA.

36. A method for identifying functional neuronal nicotinic acetylcholine receptor subunits and combinations thereof, said method comprising:

(a) introducing at least one DNA according to claim 10, or RNA complementary thereto, and DNA encoding at least one alpha subunit of a neuronal nicotinic acetylcholine receptor, or RNA complementary thereto, into eukaryotic cells; and

(b) assaying for neuronal nicotinic acetylcholine receptor activity in cells of step (a), wherein the activity is mediated by a receptor containing one or more of the subunits encoded by said introduced DNA.

37. Isolated mRNA encoded by the DNA of Claim 1.

38. Isolated mRNA encoded by the DNA of Claim 10.

39. Cells containing mRNA according to claim 37.

40. Cells containing mRNA according to claim 38.

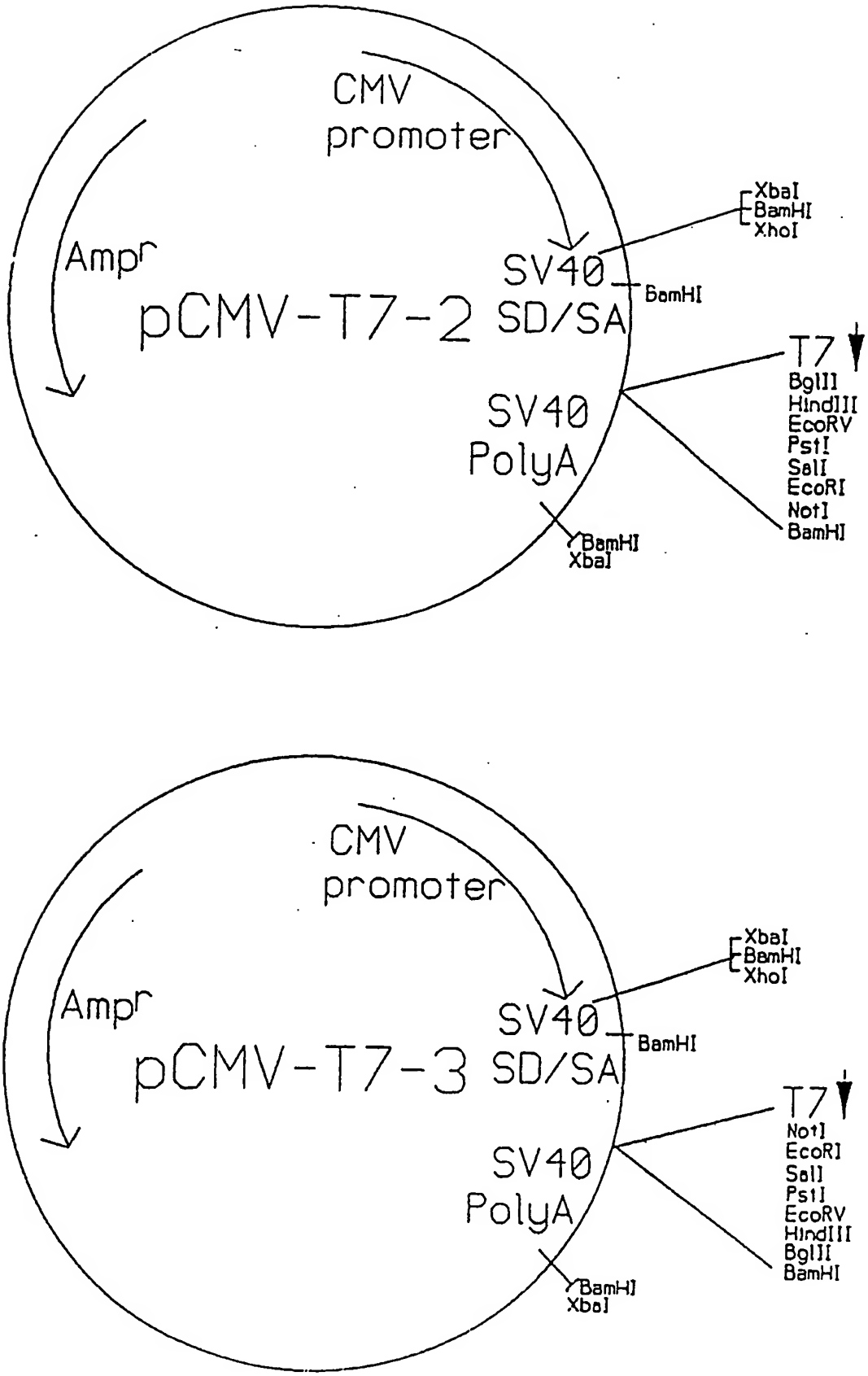
41. Cells according to claim 39 wherein said cells further contain mRNA encoding a beta subunit of a human neuronal nicotinic acetylcholine receptor.

42. Cells according to claim 40 wherein said cells further contain mRNA encoding an alpha subunit of a human neuronal nicotinic acetylcholine receptor.

43. An antibody generated against the protein of claim 29 or an immuogenic portion thereof.

44. An antibody generated against the protein of claim 32 or an immuogenic portion thereof.

FIGURE 1


CMV
promoter
Ampr
pCMV-T7-2 SD/SA
SV40
BamHI
SV40
PolyA
BamHI
XbaI
XbaI
BamHI
XhoI
T7
BglII
HindIII
EcoRV
PstI
SalI
EcoRI
NotI
BamHI
CMV
promoter
Ampr
pCMV-T7-3 SD/SA
SV40
BamHI
SV40
PolyA
BamHI
XbaI
XbaI
BamHI
XhoI
T7
NotI
EcoRI
SalI
PstI
EcoRV
HindIII
BglII
BamHI